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A STUDY OF THE FACTORS AFFECTING HEIGHT GROWTH OF LONGLEAF PINE SEEDLINGS

by

Robert Max Allen

Department of Forestry
Duke University

Date: ____________________

Approved:

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A dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of
Philosophy in the Department of Forestry
in the Graduate School of Arts and
Sciences of Duke University

1958

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Robert M. Allen

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A STUDY OF THE FACTORS AFFECTING HEIGHT
GROWTH OF LONGLEAF PINE SEEDLINGS

INTRODUCTION

Longleaf pine (Pinus palustris Mill.) is unique in its growth habit. Under normal forest management, longleaf seedlings do not start making active height growth until they are three to five years in age. In this early stemless stage the seedlings look like a tuft of grass, whence the term "grass stage."

During this period of retarded growth the seedlings can be killed by the brown spot needle blight, fire, or the piney woods hog. Attainment of merchantable size is, of course, delayed.

Since longleaf pine is a very important timber species in the South it would be very desirable if some way could be found to overcome this dormant condition. It is not likely that this problem can be solved until we know what the physiological processes are that result in the dwarf-growth habit.

This work was undertaken to investigate some of the factors which conceivably could cause this condition. The primary purpose was to provide a starting point for additional work which eventually will lead to an understanding of the causes of "seedling dormancy." Of course, it would have been a very satisfying achievement if the present work had resulted in a method of stimulating height growth but this would certainly have been a fortuitous accomplishment considering how little is known about the complex interactions which result in growth.

The present investigation was limited almost entirely to very young seedlings. This was done because it seemed especially desirable to attempt to learn about possible causes of failure of the epicotyls to elongate as do epicotyls of comparable aged loblolly and slash pine seedlings.

LITERATURE

The literature contains very little specific information on the longleaf "seedling dormancy" problem. There have been many empirical studies dealing with longleaf height growth as it normally occurs, i.e. on older seedlings, but the author has found no published reports on work with very young seedlings. The literature on longleaf height growth in the older seedlings will not be reviewed here, but for that the interested reader is referred to Wahlenberg (42), Pessin (33), Wakeley (43), and Allen (2).

Retarded seedling growth has received some attention from physiologists in the recent past. By using embryo excision techniques, Flemion (11, 12) obtained dwarfed seedlings from dormant unstratified seeds of a number of species of rosaceous plants. Dwarfed peach seedlings, for example, were characterized by shortened internodes and short, thick, green leaves. This dwarfed habit persisted until some condition such as a cold treatment was given whereupon normal growth resulted. Recently, Barton (3) has demonstrated that the dwarfed condition in seedlings from non-after-ripened embryos of _Malus arnoldia_ could be overcome by applications of gibberellic acid.

Auxin production in seedlings of dwarf maize was studied by van Overbeek (45). He found there was no difference in the auxin content of the seed of normal and _nana_ (dwarf) maize. Six-to-seven-day-old _nana_ plants produce as much auxin as normal plants. However, the amount reaching the growing zone is much less. _Nana_ plants destroyed 92 percent of the exogenously supplied indoleacetic acid compared to 37 percent for normal plants.

In working with _Ginko_, Gunckel, et.al. (17), showed that both long and short shoots have a flush of auxin when the buds start to open but the long shoots produce a second flush when elongation occurs which is not duplicated by the short shoots. It proved possible to produce a shoot reversal, particularly in the first and second laterals by decapitating the terminal shoot (18). The laterals could be maintained in the short-shoot condition on decapitated shoots by applying napthaleneacetic acid to the cut surfaces. This was interpreted to mean that the terminal bud inhibits the development of laterals but does not inhibit their opening as short shoots.

Because many aspects of growth are known to be influenced by auxins, much of the present work deals with the auxin physiology of the pines. The literature on auxins is voluminous and no attempt will be made to review it here except that which specifically pertains to pine and to the work as it was conducted.

In spite of all that has been done with auxins in non-woody and woody plants we know very little about the auxins in pine. Fransson (13) studied the auxin in _Pinus sylvestris_. In comparing pine auxin with B-indolylacetic acid, he found differences in the pH response curves and diffusion coefficients. He also demonstrated that pine auxin loses all activity when boiled with IN HCl and loses approximately 70 percent of its activity when boiled with 1N NaOH.

In 1937, Zimmerman (46, as cited by Fransson, 13) demonstrated the presence of auxin in *Pinus strobus*. Later, Mirov (29) demonstrated the presence of auxin in *P. ponderosa* and *P. torreyana* using the *Avena* curvature test. He found that in ponderosa pine the hormone concentration is higher in fast growing trees than in slow ones.

About all that can be inferred from the literature is that pines do have auxin but it is probably different from the indoleacetic acid commonly found in herbaceous species. In efforts to stimulate dwarfs of non-woody species into making normal growth by applying growth regulators there has been little if any success, except with gibberellic acid. Von Abrams (1) obtained a 30 percent increase in growth of etiolated dwarf peas at a concentration of indoleacetic acid which inhibited the tall variety. Brian and Hemming (8) also increased the growth of dwarf peas slightly by spraying with an aqueous solution of indoleacetic acid. However when suitable doses of gibberellic acid were applied to the dwarf peas they had a growth rate comparable to that of tall varieties. Several genetic dwarfs of corn have been made to grow normally by making repeated applications of gibberellic acid (Phinney, 34).

The most typical response to gibberellins is stem elongation. Marth, Audia and Mitchell (26) give the response of a considerable number of species to applications of gibberellic acid. One oak and several maples showed increases in stem length up to 400 percent over untreated controls. Loblolly pine showed some response to gibberellic acid at the highest concentration, 400 ppm, as did Virginia pine. White pine failed to show any additional growth in their tests.

Most of the tests being reported in the literature at the present time are simply recording the responses of various plants to applications of gibberellic acid. When more is known about the action of gibberellic acid we should have a much better insight into the growth processes of plants. An excellent review of the literature on the gibberellins is given by Stowe and Yamaki (40).

METHODS

Seedling Characteristics

Since there was very little information available, it was necessary to investigate many aspects of early seedling growth. One of the first questions to be answered was how does the growth pattern of longleaf pine seedlings differ from that of other seedlings? True, Huberman (20) has presented growth data for nursery-grown longleaf, loblolly, slash and shortleaf pines over an 11-month period. While his data give a general picture of comparative development, more detailed measurements of very young seedlings were wanted.

Growth Studies

The early development of longleaf, slash and loblolly pine seedlings was followed. Seeds, approximately 25 to the pot, were sowed in five-inch paper pots filled with a sandy loam potting soil. This was done in June and the pots placed in a lath house which provided 30 percent shade. Pots were kept well watered by sub-irrigation. One week after germination the seedlings were thinned to the five largest. This gave very uniform plants.

Measurements were started one week after germination. Three pots of each species were chosen at random and the seedlings carefully lifted. Measurements taken included the length of the tap root, length of the hypocotyl and length of the epicotyl. Epicotyl length was obtained by cutting the seedling transversely at the base of the cotyledons with a razor blade and then splitting the apical portion longitudinally. The distance from the base of the cotyledon to the tip of the apical meristem was termed epicotyl and measured to the nearest 1/100 inch with a ruler.

The roots were then cut off at the ground line and the green and dry weights of the tops and roots obtained. The values recorded were totals for fifteen seedlings.

Since it was not possible to follow root development too well in these small pots, a glass-sided box was constructed so that day-by-day root extension could be accurately measured. This box was constructed with the glass side at a 70° angle. The glass side was covered with a light-tight removeable cover which was kept in place except when the daily measurements were made. It was filled with the same type soil used in the pot tests. Slash and longleaf seeds were sown in the box in June. The stand was thinned to five seedlings of each species three days after the seedlings appeared to be established.

The length of the tap root and total height was measured daily on these seedlings starting two days after germination.

Longleaf and slash seedlings also were grown under continuous light and in complete darkness to compare the differences in development between the two species. The seeds were sown in moist vermiculite and placed under continuous fluorescent light for three days. Longleaf seed will germinate promptly in continuous darkness but slash pine seed will not. No visible germination occurred during a three-day period in the light. The seed was then moved into a dark cabinet and kept in continuous darkness for 28 days after germination at which time they were measured.

Photosynthesis and Respiration

A check was made on the photosynthetic and respiration rate of longleaf, slash and loblolly seedlings using an infra-red gas analyzer. Use of this machine is ably described by Bourdeau (9) and Schroder (37) consequently only the general procedure and some details of the test will be given here.

Newly germinated seedlings of each species were transplanted to one-quart plastic pots and were grown in the greenhouse in the same manner as those described in detail in the next section. Five seedlings were in each pot with two replications of each species.

After the seedlings were four months old, the surface of the soil was sealed by pouring melted wax over it to a depth of 0.5 cm. Injury to the hypocotyls by the hot wax was prevented by first surrounding them with thick layers of non-phytotoxic gum. Drain holes in the bottom of the pots were plugged with gum. Sealing was necessary because the entire pot was put into the measurement chamber and any CO_2 from the soil would have interfered with the readings. It was necessary to aerate the soil by breaking the wax seal around the top and forcing air up from the bottom of the pot with an air hose. This was done frequently and the only time the pot was completely sealed was during the time of actual measurement.

Photosynthesis was measured at 25°C and at 5,000 foot candles of light. Respiration measurements were at the same temperature. The seedlings were kept in the greenhouse until 30 minutes before measurements were due on a particular pot. The pot was then placed in an outer chamber of the apparatus for at least a 20-minute-equilibration period while measurements were being made on seedlings already in the inner chamber. About 15 additional minutes equilibration were given in the inner chamber while necessary adjustments were being made on the apparatus before measurements actually started. Duplicate sets of measurements were made on each pot.

Briefly, the measurements were made in a closed system through which outside air was first pumped. The air was kept circulating through the system by pumps and in the measurement chamber by a small fan. The photosynthesis measurements recorded the time it took for the seedlings to bring the CO_2 content from +5 to -5 percent of normal air (-5 to +5 for respiration) as measured by the CO_2 sensitive infra-red gas analyzer. Since the volume of the system was known it was possible to calculate the amount of CO_2 absorbed or given off.

Experiments on Stimulation of Height Growth

A large variety of tests were made to see if the longleaf seedlings could be stimulated into making some epicotyl elongation. If a treatment could be found which would cause even a little elongation, it would give an indication as to what to study.

These tests were of two general types--in one, the environmental conditions were varied and in the other, various chemical treatments were imposed upon the seedlings.

Except where specified, the longleaf seed in these tests came from Harrison County, Mississippi. The slash seed came from Pearl River County, Mississippi and the loblolly seed from the Talladega National Forest in Alabama.

The longleaf seed was collected from trees resembling longleaf i.e. it was not collected from trees with Sonderegger* characteristics.

In most of the tests the longleaf seedlings were germinated in a sand bench in the greenhouse by sowing them on the surface of the sand and covering them with cloth. These seedlings were then lifted and transplanted to pots when the roots were two to three inches long. Pine seedlings transplant very easily at this stage and in all of the tests described here not one seedling died from what could be attributed to transplanting. Five seedlings were in each pot. In some of the tests the seeds were sown directly into the pots and then covered with cloth in the manner described above.

The pots used in these tests were quart-sized freezer containers, paraffin-coated cardboard containers or plastic pots. Holes were made in the bottoms to facilitate drainage. In tests conducted at Duke University, the potting medium was a local North Carolina product called "builders sand" which contained a fairly high percentage of silt. The seedlings were fertilized weekly with a commercial water-soluble fertilizer according to the manufacturer's recommendations.

These tests were conducted in the greenhouse starting in the fall of 1955 and continuing through the spring of 1957. During the winter months the house had a minimum night temperature of about 15°C. Day temperatures of course varied, occasionally rising above 35°C. No tests were conducted inside during the summer months, but some were carried on outside.

The seedlings were grown under 15-hour photoperiods in these tests. Two hundred watt Mazda lamps suspended four feet above the pots were controlled by an electric timer. Additional light was provided both in mornings and evenings.

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* The so-called Sonderegger pine is a natural hybrid between longleaf and loblolly pine.

Damping-off fungi caused considerable difficulty, particularly with the longleaf which seemed to be more susceptable to this trouble than did the slash and loblolly. In some of the tests the seeds and soil were treated with an organic mercurial preparation, "Semesan" according to the manufacturers directions. This did not prove satisfactory. Evidently the fungicide is quite toxic to pines because where it was used, a high proportion of the radicles of the germinating seeds failed to penetrate the soil and grew along the surface. Where they did penetrate the soil, root growth was slow. Chemical treatment was therefore discontinued. Damping-off troubles were reduced by changing the soil when a new lot of seedlings were being germinated and by being extremely careful not to water too heavily.

The south Mississippi tests were conducted outside during the summer and fall of 1957. General procedures were the same as described above.

The only measurement taken during most of the tests was epicotyl length. These were made sometime after the secondary needles had appeared.

Because there were so many individual tests the details of each are recorded in Appendix I rather than here. There were five tests in which the seed or seedlings were subjected to different environmental conditions such as changes in photoperiod and cold treatment. There was also one test involving needle pruning and one where phloem blocks were used.

The remainder of the tests which are described in Appendix I involve the application of various growth regulators to the seedlings. Indoleacetic acid (IAA), indolebutyric acid (IBA), napthaleneacetic acid (NAA), beta napthoxyacetic acid (NOA), para chlorophenoxyacetic acid (CPA), 2,3,5 triiodobenzoic acid (TIBA), alpha (1-naphthylmethylsulfide) propionic acid

(NMSP), 2,4-dichloroanisole (DCA), kinetin (K), gibberellic acid (GA), benzotriazole (TB), 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), trans-cinnamic acid, indole carboxcylic acid (ICA), coumarin, and indole were all applied to seedlings in various combinations and rates. The most common procedure employed was to dissolve the growth regulator in alcohol and apply it to the vicinity of the terminal meristem in 5 or 10 ul aliquots.

Amino Acid Chromatography

A comparative study was made of the amino acids in young longleaf, slash and loblolly pine seedlings.

In this study the seeds of the three species were soaked in tap water for six hours, then spread out on a moist paper toweling in covered dishes. The dishes were placed under a fluorescent light at room temperature during the day and in a refrigerator at night. This regimen was followed through three complete cycles, after which samples of longleaf and slash seed were taken and the embryos excised. The entire embryo of slash pine was dropped into a 40 percent methanol solution. As soon as sufficient embryos were obtained they were boiled for one minute and then placed in a deep freeze until needed. The longleaf embryos were broken up into three sections--cotyledons, hypocotyl and apical meristem regions, and the root region. These separate samples were placed in 40 percent methanol and boiled in the same manner as the slash pine embryos.

The slash and longleaf seed not dissected for embryos along with the loblolly seed was sowed in the nursery. After several days when germination had started (when the radicles were about 0.5 cm long) samples of the longleaf and slash seed were taken. The germinating embryos were removed from the seed and the cotyledons broken off leaving the apical meristem region, along with 2 mm of the hypocotyl. These seedling tips were dropped into

40 percent methanol as soon as they were prepared and when a sample of sufficient size was obtained they were boiled. Following boiling, the tips were removed from the liquid, blotted dry on absorbent tissue, weighed to the nearest milligram, and returned to the original liquid. The samples were then placed in a deep freeze for storage.

Thereafter samples of seedlings were obtained each week and processed in the manner just described. On the older seedlings the primary needles were removed as well as the cotyledons. This was accomplished by breaking off each needle individually. Where a clean break was not obtained the needle base was removed with a sharp scalpel. As many of the embryonic needles were removed as possible taking care not to remove the apical meristem.

Weekly samples of loblolly seedlings were obtained starting two weeks after germination. Sampling was continued on all three species until the epicotyls on the slash and loblolly were over 1 cm long and the longleaf seedlings were producing secondary needles.

When all of the samples had been collected the extracts were prepared in the following manner:

> Tissue was ground in a mortar with the original liquid and placed in centrifuge tube. All washings were with distilled water which were also put into the tube with the macerated tissue.
> The extract was centrifuged and the supernatant was decanted. The tissue was resuspended in water and re-centrifuged. This supernatant was decanted into the first.
> The liquid was filtered.
> The liquid was shaken with an equal volume of ethyl ether and the ether layer removed and discarded.
> The water layer was evaporated to dryness under air stream.

> The residue (containing amino acids) was dissolved in water at a rate equivalent to 2 mg of original tissue per microliter for the slash and loblolly pine extracts and 1 mg of original tissue per microliter for the longleaf extracts. The samples were then ready for chromatographing.

The amino acids were chromatographed using the two-dimension, descending technique. Solvents employed were 71 percent aqueous phenol in the first direction and n-butanol:propionic acid:water (equal volumes of 96 percent n-butanol and 44 percent propionic acid) in the second direction. Chromatograms were run on 18 X 22-inch Whatman No. 1 paper.

The atmosphere of the chromatographic cabinets was saturated with vapors of the solvent used in the troughs and the papers were placed in the cabinets at least five hours before adding the solvent. This procedure resulted in quite uniform movement of the solvents in the paper.

After chromatographing and thorough drying, the papers were sprayed with a ninhydrin spray (95 ml ethanol, 5 ml collidine, 0.5 gm ninhydrin) and the colors developed by heating the papers in a forced draft oven at 75°C. The amino acids in the extracts were identified by co-chromatography with known acids. Several other amino acid extracts were made but since the procedures used were the same as described here they will not be elaborated upon at this time.

A test was made for sugars in these same extracts by running the papers in the same solvents and spraying with a p-anisidine reagent (6).

Radioactive Substrate Feeding Tests

The first of these tests was simply to see if materials applied to the leaves of longleaf and slash pine seedlings were absorbed and translocated.

One longleaf seedling and one slash seedling were used in this test. These were grown in pots like those previously described in the auxin tests. The secondary needle initials had just started to appear on the longleaf seedling. The epicotyl of the slash seedling was about 1 cm in length. The two basal primaries of each seedling were placed in a tiny cup holding 20 ul of a water solution of D-glucose-U-C^{14}. Total activity of the solution was one microcurrie.

The needles remained in the solution for 24 hours after which they were lifted and a radioautogram made of the separate parts i.e. needles, cotyledons, epicotyl, and roots. This was done by mounting the seedling parts on a piece of blotting paper and placing a sheet of No-Screen X-ray film on top. The entire package was then put into a light-tight holder and held for about two months before developing the film.

Longleaf and slash pine seedlings similar to those described above were used in the second test. The needles were removed with a scalpel under a dissecting microscope. The epicotyls of the longleaf seedlings were cut off just above the cotyledons. Only the apical 5 mm of the slash pine epicotyls were used. As each tip was prepared it was dropped into an 0.1 strength White's mineral solution (44). The tips were held in this solution for 18 hours at 5°C before using.

The tips were incubated in a radioactive glucose and radioactive succinate solution. The substrate was prepared by placing 2 uc of D-glucose-U-C^{14} in 0.25 ml of water in a small vial. Duplicate samples were made. The succinate substrate was made up in the same way except that 4 uc of succinic 2,3,-C^{14} acid was used per vial.

The tips were incubated in the dark at 22°C for 6, 12 and 24 hours in these two substrates. At the end of the required incubation period the duplicate samples of each treatment were composited and washed in distilled water for 30 seconds. The slash tips were separated from the longleaf tips and killed by boiling in 40 percent methanol for one minute. These tips were then macerated and extracted in the same manner as given above for the amino acid extracts except the ether-shaking step was omitted.

The extracts were chromatographed using two-dimension chromatography with the phenol in one direction and butanol-propionic acid in the other. The extracts were applied to the paper at a rate equivalent to 20 mg of original tissue weight. The slash extract was chromatographed on Whatman No. 1 paper and the longleaf on 3MM paper. This was necessary because of the large amount of non-labelled amino acids in the longleaf extract.

The chromatograms were exposed to No-Screen X-ray film for several months to locate the labelled compounds. The amount of radioactivity contained in each compound was determined by counting the area directly on the paper with a Geiger-Muller tube.

The amino acids and sugars were identified by their Rf values and by spray tests. The organic acids were identified by Rf values alone.

Auxin Chromatography

As was mentioned earlier we know very little about the growth regulating substances in pine. Since such substances play such an important role in the development of many plants it was necessary to learn something about auxin and inhibitor distribution in longleaf pine. There was the possibility that the dwarf growth habit of longleaf pine exists because it differs from the other pines in the absence of a growth promoting substance or the presence of an inhibitor.

Most of the extracts were made of young seedlings from four to eight weeks of age. Cotyledons and the needles of longleaf pine seedlings were removed and the hypocotyl broken off at the ground line. This stem portion was dropped into a container surrounded by dry ice and kept in the deep freeze until needed for extraction. Some extracts were made from the entire tops of young seedlings. Slash and the other pine seedling material was handled in the same manner. Generally, the seedlings used were those just starting epicotyl elongation.

The terminal buds of saplings, in the process of active elongation, also were used. As with the younger seedlings the buds were generally frozen as they were collected. Sometimes when dry ice was not available the material was ground and extracted as soon as possible. In all cases a decided effort was made to reduce the possibility of enzymatic breakdown of the growth substances.

The plant material was quickly macerated and placed in the extracting solvent, absolute ethanol, absolute methanol or anhydrous peroxide-free ether. Alcohol extractions were made in a deep freeze at -10 to -20°C. Ether extractions were made at 5°C. The plant material was usually extracted for three days with three solvent changes.

The extraction and partitioning procedure used was essentially that given by Kefford (27) the only major modification being when methanol or ether was used.

In brief, the partitioning was as follows:

> With ethanol or methanol extracts the alcohol was evaporated.
> The aqueous residue was acidified to pH 3.5 with 0.5N phosphoric acid and shaken, three times with peroxide-free ether. The water fraction was discarded.
> The ether fraction was shaken three times with equal volumes of 0.5 percent sodium bicarbonate. The sodium bicarbonate fraction was then washed with fresh ether. This constituted the ether fraction containing the neutral substances and was saved.

> The water fraction was acidified to pH 3.5 with 0.5N phosphoric acid and shaken three times with fresh ether. The water fraction was discarded.
> The combined acid ether fraction was washed with a small volume of dilute phosphoric acid. The remaining water was frozen out of ether fraction and the ether evaporated. The residue was picked up in ethanol. This fraction contained the ether-soluble acidic substances.

The ether extracts were first shaken with a small volume of dilute phosphoric acid and then handled in the same manner as described above starting at step three.

Only the acidic fraction was investigated in this work.

Descending chromatography was used entirely with both one-dimension and two-dimension chromatograms being made. Most of the extracts were run on Whatman No. 1 paper. Occasionally 3 MM paper was used when it was desired to run larger quantities. Number E. 17 paper was used where it was desired to obtain very large amounts for purification.

Usually the extract was spotted on the paper in the morning by applying it with a micro-pippette and drying with a stream of warm air. The origin was held to a spot of 6 mm diameter. After the papers were spotted they were placed in the tank, which contained developing solvent in the bottom, until evening. This allowed the paper to come into equilibration with the atmosphere inside the tank. The solvent was applied to the papers in the evening and chromatograms removed the following morning.

A considerable number of developing solvents were used. The one used most frequently was an isopropanol:ammonia:water (8:1:1) solvent developed by Stowe and Thimann (39). Other solvents used were isopropanol:water (8:2), acetic acid:water (1:6) and isobutanol:methanol:water (8:1:1) which was similar to that used by Nitsch (31). The unknowns were usually run with IAA as a standard.

Compounds were detected by viewing the paper under ultra-violet light (wave length 2900-3700 angstroms) and by spraying with Erlich reagent as given by Good et.al.(16) or a ferric chloride:perchloric acid reagent (21). The Erlich reagent was generally used since it was sensitive and yet not too destructive to the paper. In some instances better resolution was obtained by spraying the papers first with Erlich reagent and heating to 75°C in the regular manner, then over-spraying lightly with the ferric chloride:perchloric acid reagent.

Bio-Assay of Growth Substances

Several methods were used to check the biological activity of the compounds extracted from the pines.

A flax test which was suggested by Dr. A. W. Naylor proved helpful in locating areas of activity on the paper. In this test the origin spots were placed 3 cm apart on the chromatogram and a line drawn across the paper 30 cm from the bottom of the origin spot. The chromatogram was then run in the desired solvent until the solvent front had travelled the specified 30 cm. The paper was taken out, dried and ruled off in 3 cm squares. The fluorescent spots were determined under ultra-violet light and recorded.

The paper was then cut into the 3 cm squares and the squares placed in 2-inch petri dishes. The squares were wet with 0.5 ml of distilled water and 10 flax seeds distributed evently over the paper. At least three replications of each extract (three origin spots on the chromatogram) were made.

The petri dishes were put into moist chambers and the flax seed allowed to germinate under room conditions. Germination counts were made after two days. The roots of the germinating seed were broken off next to the seed and quickly weighed to the nearest milligram. Root growth proved to be a more sensitive criterion of growth effects than germination although the two were correlated.

The *Avena* straight growth test also was used to check the activity of the compounds isolated on the papers. In most instances, the papers were prepared in the same manner as described above with the flax. However in some of the tests just the spots that fluoresced were cut from the paper and tested. A few tests were made by eluting the compounds from the paper before testing but usually the paper sections were placed in the media.

The oats used in these tests were the hull-less variety Brighton*. They were soaked in tap water for an hour, placed on moist paper towelling in a covered dish and set in the light for three hours. The seeds were then planted in soil and placed in a dark room. After 24 hours the germinating seeds were exposed to 12 hours of low intensity red light. Twenty-four hours later the coleoptiles were from 2.5 to 3 cm long and were ready for cutting. The room where this work was done had a very poor temperature control and there was considerable variation in the length of time it took for different batches of seedlings to produce coleoptiles of the desired length. This was not considered serious, however, since the main purpose of these tests was to see whether the compound in question was an inhibitor or promotor and not to evaluate the compounds quantitatively.

Two, 5 mm sections were cut from each coleoptile. The apical 3 mm was not used. The sections were cut with three razor blades mounted in a holder so that the two sections could be cut simultaneously. It took several hours to obtain the desired number of sections so they were placed in a dish of two percent sucrose as they were cut. The sections were then thoroughly mixed before they were placed in a test solution. All of these operations were carried out under a red safelight.

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* Supplied through the courtesy of the Canadian Department of Agriculture, Central Experimental Farm, Ottawa, Canada.

The test solutions were prepared by placing the paper cut from the chromatogram in a 2-inch petri dish plus 3 ml of two percent sucrose solution buffered to pH 5.2 with a phosphate buffer. IAA controls were always included. In some of these the test solution contained 0.1 ppm IAA and in others 25 ug of IAA was placed on the paper and chromatographed like the plant extracts. In the latter just the IAA spot was cut from the paper and placed in the petri dishes.

Ten coleoptile sections were placed in each petri dish and the dishes placed in the dark for 24 hours before measurements were made. The dishes were shaken occasionally by hand in an effort to reduce curvature of the sections. The sections were measured with a ruler to the nearest millimeter.

The other test used to determine the biological activity of the compounds was a slash pine hypocotyl test. In this, slash pine seeds were first soaked in a tap water for six hours, spread out on moist paper towels in a covered dish and the dishes placed under fluorescent light for 48 - 64 hours. The seeds were then sowed in dishes of moist vermiculite and placed in the dark room. The light treatment is necessary to get prompt germination of slash pine seeds in the dark.

After the hypocotyls of the slash seedlings had elongated to about 4 cm the sections were cut. A single 5 mm section was cut from each hypocotyl. It was cut just below the cotyledons or in those seedlings with a hook in the hypocotyl, just below the hook. The sections were handled in the same manner as described above for the oat coleoptiles.

They were measured after 48 hours in the test solution. Length proved to be the most sensitive criterion. The weights were taken in several tests but they proved to be extremely variable due to differences in diameter. This could have been overcome by weighing before and after testing but facilities were not available for the earlier weighings.

RESULTS

Seedling Characteristics

The longleaf pine is unique in many respects. To begin with, the seed is very large, three to four times heavier than that of slash and loblolly, and has a very thin seed coat compared to the much harder ones of the other two. The embryo is also very large when compared to that of the slash pine. In one test, longleaf embryos from dry seeds averaged 11.0 mg each while slash embryos averaged only 2.7 mg. Eighty-one percent of the total longleaf embryo weight was in the cotyledons while the slash cotyledons made up only 48 percent of the total embryo weight.

Germination

Longleaf seed germinates very easily, fresh seed germinating in two days if temperature and moisture conditions are favorable. It shows very little if any dormancy. Loblolly seed, on the other hand, is quite slow and individual lots may be dormant. Longleaf seed will germinate at 40°F, some 5 to 10° lower than will slash and loblolly, but will not easily germinate above 80°F. Slash and loblolly seed will germinate at temperatures higher than this (43).

When slash or loblolly seed germinate the normal course of events is for the radicle to emerge and then the hypocotyl begins to elongate and lift the seed coat above the ground. When the hypocotyl has grown to nearly its final length the cotyledons begin to elongate and finally push themselves free from the seed coat. The primary needles at this stage are barely visible and do not start to elongate appreciably for several days.

Longleaf acts much differently. Hypocotyl elongation is completed shortly after the radicle emerges from the seed. In fact the hypocotyl is not a very distinct region in the longleaf. There is a color difference, however, with about 0.5-1.0 cm of the length below the cotyledons being either green or red. Marking this zone into divisions with India ink shows that there is some growth taking place as in normal hypocotyl elongation.

In longleaf the cotyledons start elongating just as soon as germination starts. They take over the function of the hypocotyl in lifting the seed off the ground. See Figure 1. The primary needles of longleaf start growing about the time the cotyledons slow down in their elongation.

Seedling Growth

The early top growth of longleaf and slash seedlings is plotted in Figure 2. The green weight increase for longleaf in the first several weeks was attributable to growth of the cotyledons and primary needles. Between the fourth and the fifth week there was a lag period when the secondary needles began to appear and a sudden spurt as they elongated. The epicotyl length in longleaf reached a maximum of about 0.15 inch by the time the secondary needles appeared and then did not increase in length in the next eight weeks that measurements were taken.

In slash pine the early growth increase is also due to the growth of

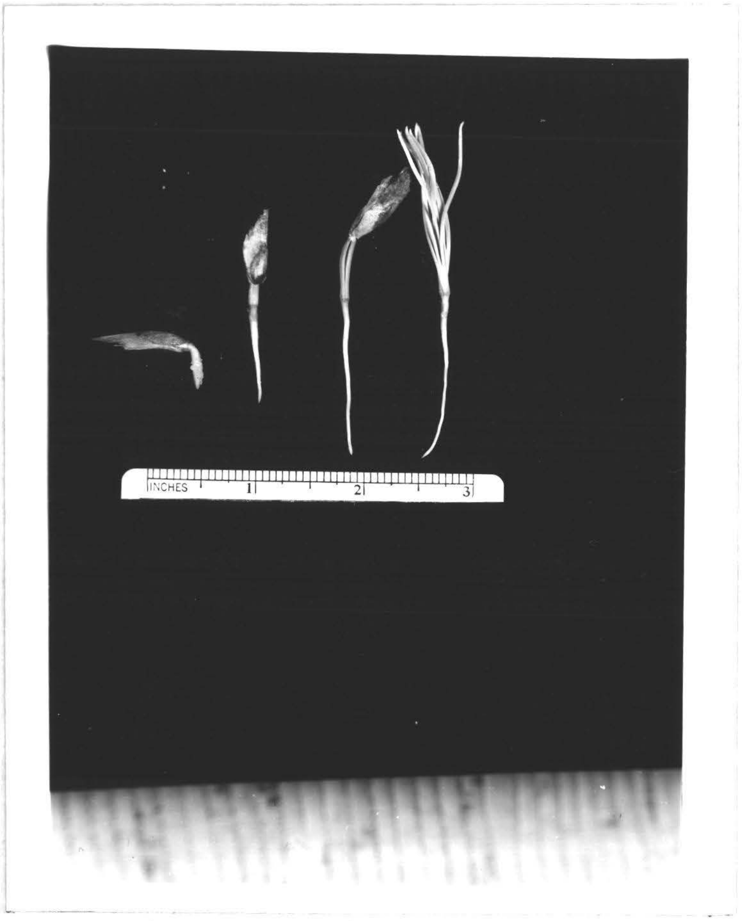
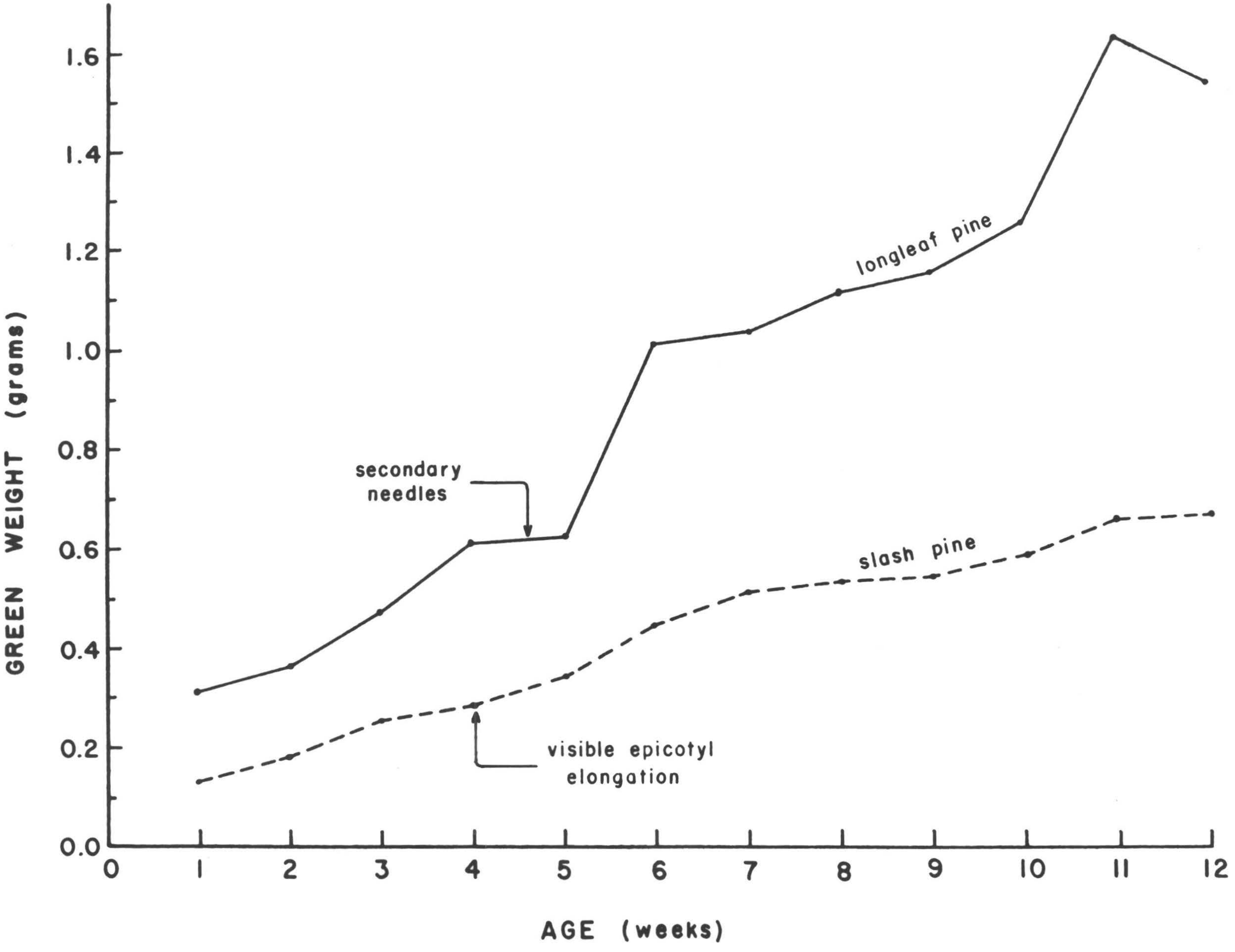


FIGURE 1. LONGLEAF PINE SEEDLINGS IN EARLY STAGES OF DEVELOPMENT

FIGURE 2. GROWTH OF TOPS OF LONGLEAF AND SLASH PINE SEEDLINGS


GREEN WEIGHT (grams)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
AGE (weeks)
0
1
2
3
4
5
6
7
8
9
10
11
12
longleaf pine
slash pine
secondary needles
visible epicotyl elongation

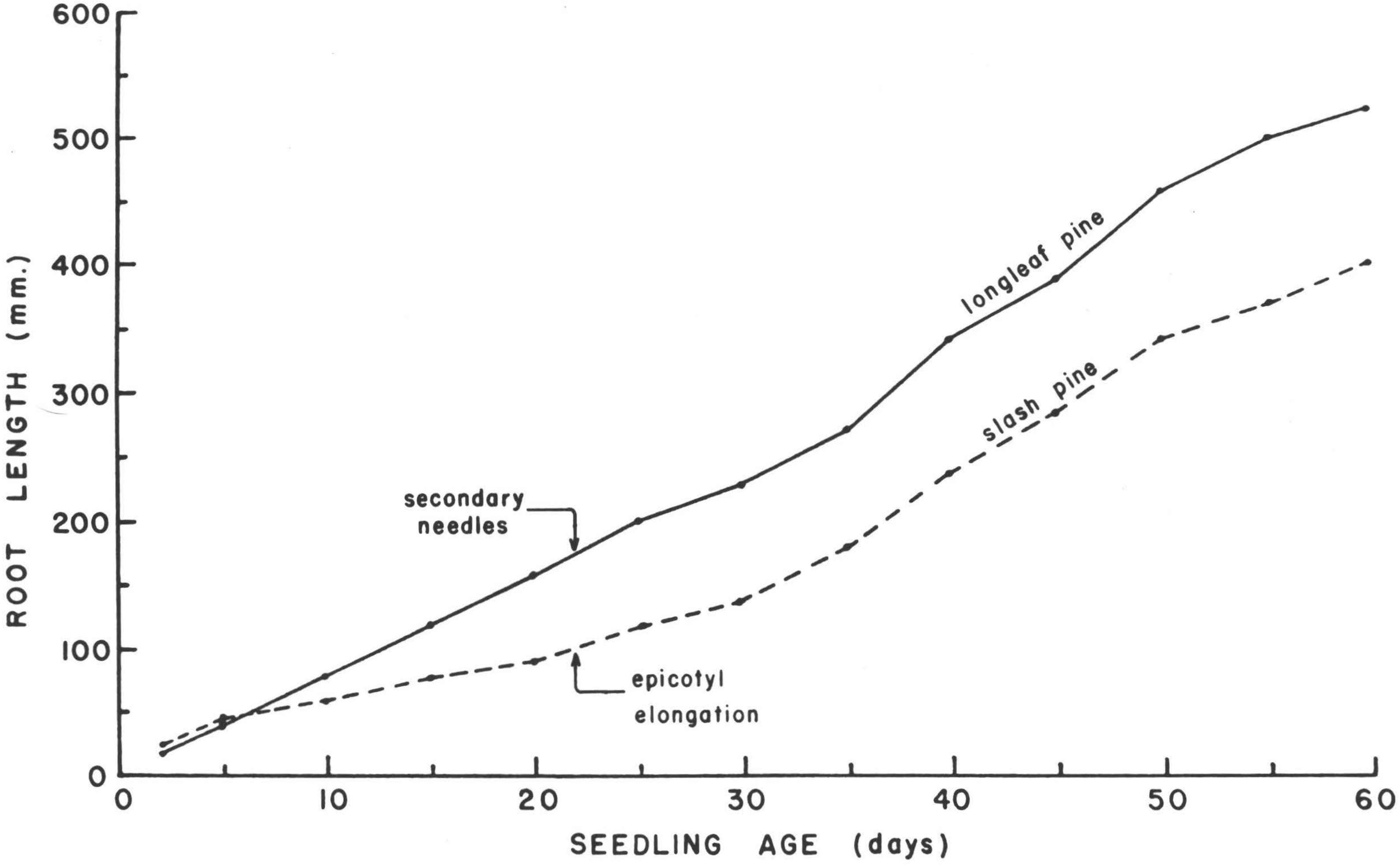
the cotyledons and primary needles. Once the epicotyl starts to elongate there is a very close correlation between epicotyl length and green weight of tops as might be expected. It is of interest to note that the epicotyl of slash starts to elongate about the same time that secondary needles appear in longleaf. This similarity was noted in many other instances where longleaf and slash seedlings of the same age are grown under similar conditions. Secondary needles were not seen on the slash seedlings during the 12 weeks they were under observation. This was not unexpected since secondary needles do not appear on nursery grown slash seedlings for the first four to five months after germination.

It should be mentioned that the measurements recorded here were taken on seedlings sowed in June in south Mississippi. Epicotyl elongation of the slash and the appearance of secondary needles on the longleaf occurred sooner than if an early spring sowing date had been used.

Root growth of longleaf and slash seedlings planted in a glass-sided box is plotted in Figure 3. These measurements represent elongation of the tap-root and do not take into account number or extent of the laterals.

Rate of growth of the longleaf tap root was very steady throughout the 60-day period of observation and showed no evidence of being affected by the appearance and development of the secondary needles. In slash pine there was a small reduction in growth rate during the period prior to elongation of the epicotyl. Under these conditions, where the box was filled with a friable soil throughout its depth, slash pine was strongly taprooted and not at all unlike longleaf (Figure 4). This is in contrast to what is generally found under nursery conditions where slash pine is much less strongly tap-rooted than longleaf pine.

FIGURE 3. GROWTH OF LONGLEAF AND SLASH PINE TAPROOTS


ROOT LENGTH (mm.)
0
100
200
300
400
500
600
SEEDLING AGE (days)
0
10
20
30
40
50
60
longleaf pine
slash pine
secondary needles
epicotyl elongation

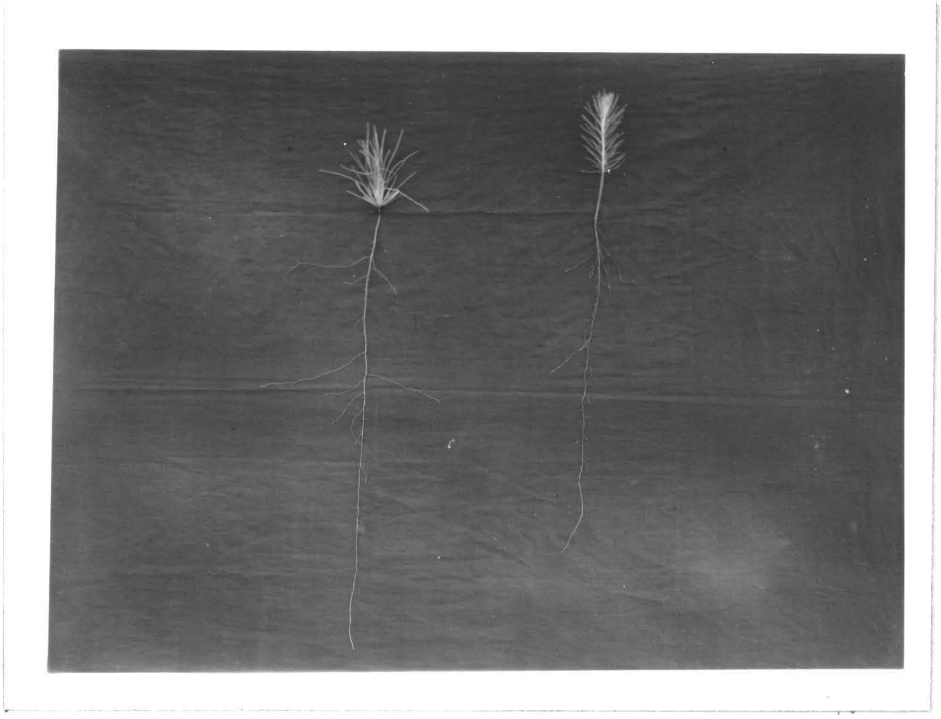


FIGURE 4. ROOT DEVELOPMENT OF A LONGLEAF AND A SLASH PINE SEEDLING

Effects of Light and Darkness

There were considerable differences between longleaf and slash pine seedlings grown in continuous light and in continuous darkness for 28 days (Table I).

In darkness the slash pine hypocotyl elongated greatly in length just as is common for many herbaceous species. At the same time tap roots of dark-grown seedlings were shorter, owing perhaps to a deficiency of food materials. Cotyledons were shorter in the dark-grown than in light-grown seedlings and the primary needles though initiated did not elongate. In contrast, though there was some increase in hypocotyl length in dark-grown longleaf seedlings it was nowhere near the degree obtained with slash. The cotyledons of dark-grown longleaf were longer than those grown in the light. The primary needles, unlike those of the slash, elongated in the dark.

The differences in cotyledon and needle growth between the slash and longleaf seedlings may be caused by differences in food reserve at the time various processes associated with development are occurring. In slash, as was described earlier, the hypocotyl growth is nearly completed before the cotyledons start elongating and primary needles do not start until several days after the cotyledons have attained near maximum length. Thus in the dark-grown slash seedlings most of the available food may have been used during hypocotyl elongation and there was not enough left to allow for cotyledon and primary needle growth. In the longleaf, since there is relatively little hypocotyl elongation and since cotyledon and primary needle growth occur earlier in the development of the seedling than in slash, there would be correspondingly more food material available at the time of elongation. An alternative hypothesis is that light is necessary to activate some physiological process necessary for the continued elongation of the slash cotyledons

Table I

EFFECTS OF LIGHT AND DARKNESS ON LONGLEAF AND SLASH PINE SEEDLINGS

Environmental conditions	Length in Centimeters			
	Hypo-cotyl	Tap root	Coty-ledons	Primary needles
Dark-grown slash	10.4	2.4	2.4	0.1
Light-grown slash	4.7	5.6	3.6	1.6
Dark-grown longleaf	0.9	5.9	7.1	1.0
Light-grown longleaf	0.6	7.8	5.5	2.1

and primary needles which is not required in the longleaf.

The fact that there was some additional hypocotyl length in the dark-grown longleaf seedlings indicates that they act in some degree like the slash, i.e. greater hypocotyl length is possible when light does not inhibit some process associated with elongation. Their failure to elongate to a length of 5 to 10 cm indicates that the short hypocotyls of longleaf are not due to light inactivation of auxin or the activation of an inhibitor by light.

Correlation Between Hypocotyl and Epicotyl Growth

It may be assumed that the physiological processes responsible for reduced hypocotyl growth in longleaf seedlings are the same or similar to those which result in dwarfed epicotyl growth. At least there is a correlation between hypocotyl length and subsequent epicotyl elongation in hybrid seedlings of longleaf.

The seedlings resulting from wind-pollinated seed collected from natural longleaf hybrids exhibit a wide range in hypocotyl length upon germination (Figure 5). Wind-pollinated seed was collected from four trees that appeared to be natural hybrids of longleaf (these trees were thought to be hybrids of longleaf and loblolly although one may be a hybrid between longleaf and slash). The pollen parent of these seeds could have been longleaf, loblolly or self. It is not likely that slash was the pollen parent because of a difference in flowering dates. Following germination, seedlings with a wide range of hypocotyl lengths were transplanted and epicotyl growth was followed. Seedlings with the longest hypocotyls were the first to show visible epicotyl elongation. Figure 6 shows that there is a strong positive correlation between hypocotyl and epicotyl length in young seedlings.

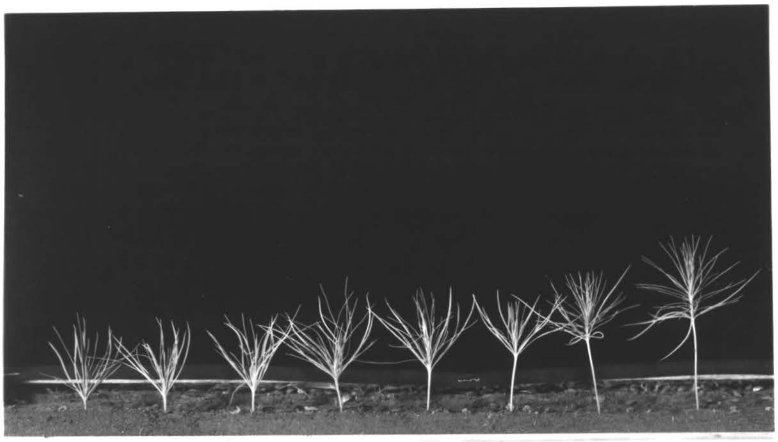
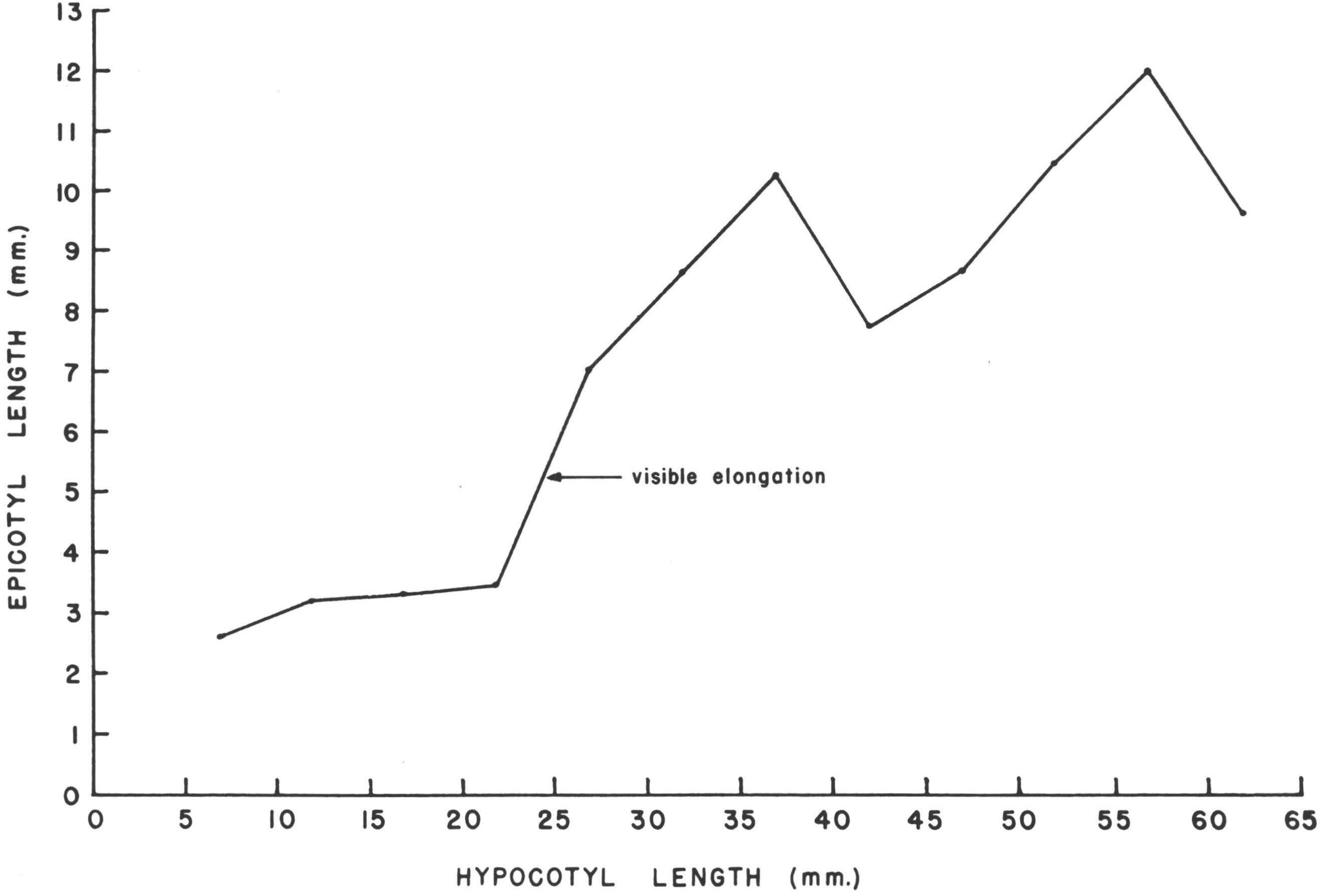


FIGURE 5. VARIATION IN PROGENY OF SONDEREGGER PINE

Epicotyl elongation has started in seedlings with longest hypocotyls.

FIGURE 6. RELATIONSHIP BETWEEN HYPOCOTYL LENGTH AND EPICOTYL LENGTH IN HYBRID PINE SEEDLINGS


EPICOTYL LENGTH (mm.)
HYPOCOTYL LENGTH (mm.)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
0
5
10
15
20
25
30
35
40
45
50
55
60
65
visible elongation

Photosynthesis and Respiration

Photosynthesis and respiration rates were checked on longleaf, slash and loblolly seedlings of the same age (Table II).

Longleaf had the highest rate of photosynthesis and respiration on a per seedling basis but the lowest rate per gram of leaf weight. But longleaf seedlings had over twice the needle weight of the slash and loblolly since the secondary needles had already formed and elongated also there was probably more mutual shading by the needles.

The apparent lower rate of photosynthesis and respiration per unit of needle tissue of the longleaf may have been due to the secondary needles making up the major portion of the needle volume in the longleaf. Bormann (7) indicates that primary needles of Pinus taeda have a higher rate of photosynthesis than secondary needles. The photosynthetic and respiration rates of longleaf needles compare faverably with the rates of 9.9 and 1.6 mg CO_2/hr. obtained by McGregor (27) for loblolly pine with secondary needles.

Observations on the relation of soil aeration to photosynthesis were made with these seedlings. The pots in which the seedlings were grown were entirely sealed off with wax and gum since the entire pot was to be placed inside the measurement chamber. This meant gas exchange between the air and soil was nil. When the first replication was run, shortly after sealing the pots, normal photosynthetic rates were obtained. When the next replication was measured several hours later the photosynthetic rate had fallen to zero on the slash and loblolly and to about half the normal rate with the longleaf. The rate on the longleaf dropped to zero after several more hours. This situation was corrected by opening the pots and pumping air into the sand. Thereafter when measurements were made the pots were kept well aerated by forcing air into the sand and sealing them just before measuring.

Table II

PHOTOSYNTHESIS AND RESPIRATION RATES[a] OF SLASH, LOBLOLLY AND LONGLEAF PINE SEEDLINGS

Species	Photosynthesis (per seedling)	Respiration (per seedling)	Photosynthesis (per gram dry needles)	Respiration (per gram dry needles)
Slash	2.3	0.7	23.1	7.5
Loblolly	1.4	0.6	17.6	7.1
Longleaf	2.8	0.8	12.5	3.7

a. Milligrams of CO_2 per hour.

Vigor

General vigor is another factor in the longleaf growth habit which should be considered. Epicotyl elongation in slash and loblolly occurs in seedlings at about the same time whether they are vigorous or not. There is, of course a difference in amount of elongation but seedlings which are very chlorotic and look as if they are about to die will show some epicotyl growth. With longleaf, however, even the older seedlings will not elongate unless they are very vigorous. Anything that reduces the vigor of the seedling whether it be grass competition, brown spot defoliation or overhead shading will prolong the "grass stage" (2). Conversely, hoeing, watering, spraying and other cultural practices which improve the vigor will shorten the grass stage. It has been possible through pampering longleaf seedlings by these practices to produce four inches of stem length in the first growing season and up to 42 inches by the end of the second growing season.

Thus, it seems that for normal elongation to occur in longleaf, a certain physiological process or processes must take place at a very high rate to produce a necessary level of factor or factors, before elongation can take place. This is mentioned to emphasize that while much of the work described here was aimed at trying to find a single factor which is responsible for longleaf growth habit, we must not lose sight of the fact that many reactions may be involved and there may be no single key compound. We hope that this is not the case.

Stimulation of Height Growth

The purpose of these tests was to induce longleaf seedlings into making epicotyl growth in order to gain some clue as to what causes the dwarf-growth habit. As was explained earlier the only measurement taken in the majority of the tests was the epicotyl length.

The tests fall into two general classes: those in which the environmental factors were varied and those in which chemicals were applied.

Effect of the Environment

Vernalizing the seed from zero to 12 weeks at 5°C and subjecting germinated seedlings to the same temperature for zero to eight weeks had no effect on epicotyl length when measured 12 weeks after transplanting (Appendix I, Tests 1 and 2).

The epicotyls in these particular tests varied from 6 to 15 mm in length on individual seedlings but there was no difference between treatments. It is felt that with this much variation among seedlings that a treatment difference would occur if there were any response of the seedlings at all. By this it is meant that if growth control were so rigidly fixed that there was no variation whatsoever among seedlings they would be much less sensitive to treatment. The fact that they can vary in epicotyl length indicates that if treated in the proper way, some response should be obtained.

In the seed vernalization experiment, a few of the seeds in the 12-week treatment had grown a little during the period they were in the cold. This may have been the result of the one day the refrigerator warmed up. When these seedlings had completed their germination, after potting, the cotyledons had an abnormal shape—much shortened, thick at the base and coming to a sharp point at the tip, not unlike the cotyledons of the seeds soaked in kinetin which will be discussed later.

The various lengths and temperatures of seed soaking (Appendix I, Test 3) produced no treatment differences in epicotyl length.

Photoperiod does influence epicotyl length. In one small test, 30 longleaf seedlings growing under a 16-hour-day had an average epicotyl length of 7.1 mm while those growing under a 9.5-hour-day averaged 5.3 mm. Initiation of secondary needles is retarded under short-day conditions. Only five out of the 30 short-day seedlings had secondary needles while they were present on all of the long-day seedlings.

In a more definitive test involving length of day and cold (Appendix I, Test 4), the epicotyls of seedlings growing under long-days again were longer than those of short-day seedlings (Table III). The epicotyl length was closely correlated with the dry weight of tops. The differences in top weight between long- and short-day seedlings was primarily due to lack of secondary needles on the latter seedlings.

The two week period of cold seemed to have little effect upon the epicotyl length.

Although significant differences in epicotyl length were obtained between long-day and short-day seedlings, they do not mean much since these differences were small and were not the result of elongation as we think of it in slash pine. It seems safe to say that long days alone will not stimulate very young longleaf seedlings into making normal height growth. Additional verification for this statement is provided in an observation where longleaf seedlings were germinated and grown under continuous light for a period of six months without normal elongation resulting.

It must be concluded from these tests with cold treatment of both seed and seedlings, that the dwarf-growth habit of longleaf pine cannot be broken by cold treatment as was done by Flemion (11) with the dormant embryos of rosaceous seeds.

Table III

EFFECT OF PHOTOPERIOD AND COLD ON LONGLEAF SEEDLINGS[a]

Treatment	Epicotyl[b] length (mm)	Dry weight of tops (gms)	Dry weight of roots (gms)
Long day	6.2	0.58	0.20
Short day	4.3	0.32	0.19
Short-long day	5.1	0.61	0.20
Long-short day	4.4	0.28	0.19
Short-cold-short day	4.4	0.35	0.17
Short-cold-long day	5.2	0.57	0.18
Long-cold-long day	6.4	0.73	0.23
Long-cold-short day	5.7	0.32	0.18

a. Average per seedling.
b. F.I. 0.05=2.5.

Since longleaf pine initiates secondary needles about the time the epicotyls of the other pine species start elongating, it was desired to see what effect the continuous removal of the secondary needles would have on the epicotyl growth of longleaf pine (Appendix I, Test 5). The primary needles were not removed.

The pruned seedlings formed a definite bud, complete with bud scales, one month after needle removal began. No such development was found on over 1,000 seedlings of the same age which were allowed to develop normally. All of these had a juvenile type bud.

In April, three months after needle removal began, 10 treated and 10 control seedlings were lifted and measured. The epicotyl length on the seedlings with the secondary needles removed averaged 10 mm compared to 7 mm for the intact seedlings. The green weight of the tops of the intact seedlings averaged a little over twice that of the pruned seedlings.

The unpruned seedlings set winter buds in August. The pruned seedlings set dormant buds four weeks later. The seedlings were placed under a 15-hour-day (from a normal day) in early November. The buds started to open on the pruned seedlings by December 1, but it was mid-January before the buds on the unpruned seedlings began to break.

The remainder of the seedlings were lifted when they were 16 months old. Epicotyl length of the seedlings which had been needle pruned averaged 19 mm (range 12-27 mm). There was no visible internodal elongation in the seedlings of either treatment. The difference was due simply to the fact that a new set of secondary needles were laid down each time a set was removed. This resulted in the pruned seedlings having many more nodes than the unpruned ones.

Thus it appears that the secondary needles alone are not responsible for the growth habit of longleaf pine. It had been proposed prior to this test that the secondary needles might prolong the dwarf condition by producing a substance that inhibited epicotyl elongation or compete with the epicotyl for some substance necessary for its elongation. This still may be true but this test indicates that it is not just the secondary needles that are responsible for the growth condition. If the premise were correct, then the cotyledons and the primary needles also contribute to the cause.

It is also very doubtful that the roots produce any inhibitory substance necessary for epicotyl elongation. There were no differences in epicotyl length between seedlings with the phloem blocks (Appendix I, Test 6) installed just below the cotyledons and the untreated controls. Additional evidence is furnished by grafting experiment in which longleaf seedlings in the cotyledon stage were grafted into the apical buds of one-year-old slash seedlings. These seedlings developed in a manner normal for longleaf. That the grafting did not seriously upset the longleaf metabolism is evidenced by the fact that some of these seedlings were making height growth after two growing seasons. This is normal for longleaf seedlings which receive excellent care.

It may also be surmised from this test that the slash needles do not produce any substance that is translocated upward which will cause early elongation of the longleaf epicotyl. Neither did the longleaf seedling produce any substance which inhibited the growth of the lateral branches on the slash stock. In fact, sprouts arose from the stem of the slash stock after all the branches were removed.

Doubling the oxygen concentration of the air did not cause any appreciable increase in epicotyl length over those growing in normal air (Appendix I, Test 7). The average epicotyl length for longleaf seedlings growing in enriched air and normal air was 3.4 mm and 2.6 mm respectively; slash seedlings averaged 11.2 mm and 5.9 mm respectively. These differences might be due in part to the fact that the inside of the box in which the seedlings were growing was on the average about 1°C warmer than the outside air where the control seedlings grew.

Based on the information gained from these tests, plus the fact that there are no recorded observations of seedlings making active epicotyl elongation in the cotyledon or primary needle stage, it appears that longleaf cannot be stimulated into making early height growth by varying the normal environmental factors. Of course, it is possible to shorten the three-to-five-year period usually required for seedlings to begin height growth by varying the environment but this is accelerating the normal processes and not actual stimulation.

It is very unlikely that mineral nutrition is responsible for the dwarf-growth form of longleaf pine seedlings. Longleaf seedlings have been grown under almost every conceivable mineral regime in tests (25) as well as under widely varying fertilizer practices in the nurseries in the South and there has not been any recorded instance of seedlings in the primary needle stage making active epicotyl elongation.

The evidence therefore points to some internal mechanism (s), endogenously controlled which accounts for the longleaf growth habit.

Chemical Treatments

The purpose of these tests was to induce the longleaf seedlings into making active epicotyl elongation by applying various growth-regulating substances.

In the first of these tests, longleaf seed were soaked in various concentrations of IAA, IBA and NAA (Appendix I, Test 8). When these seedlings were measured five months after treating, no differences were found in the length of the epicotyle that could be attributed to treatment. NAA at a concentration of 1,000 ppm caused some epinasty in the cotyledons and a swelling at the point of their attachment. The needles were stunted but the epicotyls averaged as long as those in the untreated controls. This high concentration did cause quite a proliferation of lateral roots.

Soaking the seeds in different concentrations of TIBA, DCA and NMSP (Appendix I, Test 9) had no effect upon the epicotyl length of the seedlings three months after treating. There was some indication that soaking the seed in TIBA at 100 ppm for 24 hours increased the epicotyl length over that of untreated controls (10.2 mm vs. 8.8 mm) but the difference was not statistically significant nor was it borne out in the following test.

There were no differences that could be attributed to treatment in root, cotyledon, or primary needle length in seedlings from seed that had been soaked in IAA, GA or TIBA at concentrations of 4, 20, and 100 ppm and grown in continuous darkness (Appendix I, Test 10). Kinetin at 4 ppm had no apparent effect but at 20 ppm had an adverse effect on seedling growth. The average length of the cotyledons was 3.6 cm compared to 5.7 for the untreated controls, while the length of the primary needles averaged 0.3 cm and 0.8 cm respectively. At 100 ppm K, there was no germination.

In seedlings given the same seed treatments but grown in the light there was no apparent treatment effect on the length of the cotyledons, primary needles or epicotyl except for the K treatments. Here again 4 ppm seemed to have no effect but at 20 ppm a reduction in cotyledon and primary needle length was observed. The epicotyl length averaged 1.3 mm for the

K-20 treatment compared to 1.8 for the untreated controls. There were a few seed which germinated in the K-100 treatment. These had very short cotyledons which were thick at the base and tapered to a sharp point. They looked very much like the seedlings described in the vernalization test. The K-100 seedlings showed signs of recovery with time (Figure 7).

The treatments in these seed soaking tests showed little promise of causing epicotyl elongation in longleaf seedlings. This may have been because the interval between treatment and time epicotyl elongation should occur (in comparison with slash) was several months. In this time the growth regulator compound could have been immobilized in some way within the seedling so it should be ineffective in stimulating any elongation. For this reason the effort was concentrated on treatment of seedlings.

The growth regulators were applied in aqueous solutions in the first tests on longleaf seedlings. These were applied either by dipping the tops into the solutions or by spraying the seedlings until run-off occurred.

In two tests where IAA was sprayed on the seedlings (Appendix I, Tests 11 and 12) there were no treatment responses as measured by the length of the epicotyl or number of secondary needles on four-month-old seedlings.

In another test the seedlings were dipped in aqueous solutions of IAA, NAA, NOA and CPA at concentrations of 1, 10, 100 and 500 ppm (Appendix I, Test 13). The cotyledons of these seedlings exhibited a considerable amount of epinasty one week after treating. Those treated with NAA and CPA showed the most epinasty with NOA being intermediate between the IAA and NAA. One month after treating, 80 percent of the NAA and CPA seedlings treated with 500 ppm were dead as were 20 percent of the NOA seedlings. All of those treated with IAA were alive.

When the epicotyl lengths were measured three months after treatment

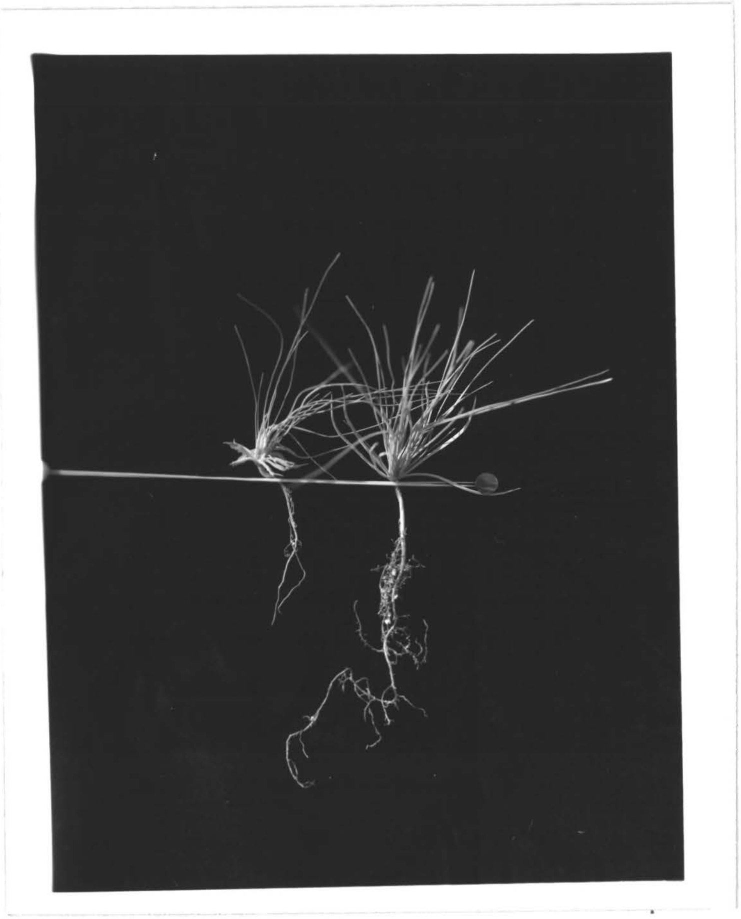


FIGURE 7. EFFECT OF KINETIN ON LONGLEAF PINE SEEDLING

Left—treated. Right—control. Note cotyledon shape on treated seedling.

there were no differences that could be attributed to the treatments. No measurements were made on seedlings treated with the highest rates of NAA and CPA as most of them were dead. It was expected that there would be differences in epicotyl length between treatments in this test. The primary needles of the NAA-100 seedlings for example were very twisted showing powerful auxin effects but the epicotyl length averaged about the same as that of the untreated controls, 4.6 mm vs. 4.4 mm respectively.

Dipping the tops of seedlings into aqueous solutions of kinetin at concentrations of 0.01, 0.1, 1 and 10 ppm had no apparent effect (Appendix I, Test 14). There was no visible effect upon the cotyledons or primary needles during growth nor upon the length of the epicotyl when the seedlings were measured, three months later. This may have been because the kinetin did not get into the needles. Kemp et. al. (22) reported that foliage sprays at concentrations of 100 ppm produced no observable results on several species (presumably sunflower and bean although it was not specifically stated).

There were no differences in epicotyl length attributable to treatments shown by seedlings whose tops were dipped in aqueous solutions of TIBA, NMSA, TCA and DCA (Appendix I, Test 9). In fact, the only observable response was to 1,000 ppm TIBA where the tips of the cotyledons showed a slight curling and necrotic spots.

It was thought that the negative results obtained in these tests might be due in part to the substances applied to the seedlings not getting into the needles. This, of course, was not the case in some of the tests where there was considerable epinasty shown by the cotyledons and primary needles. After some preliminary testing it appeared that applying the compounds in alcoholic solutions would get more of the material inside the plant. Therefore in the later tests the growth-regulators were applied to the vicinity of the apical meristem in 5 or 10 ul aliquots of 95 percent ethanol.

In a test of a variety of growth regulators, IAA, TIBA, K, GA, 2,4,6-T and BT (Appendix I, Test 15), there appeared to be some slight stimulation in longleaf epicotyl elongation with K, TIBA, 2,4,6-T and GA (Table IV).

This is a curious assortment of compounds for all of them to promote elongation in longleaf. Kinetin is a cell-division factor (28) while the GA effect is primarily in increasing elongation although there are reports of it increasing cell division (35). The 2,4,6-T is generally considered an anti-auxin (19) while several effects have been ascribed to TIBA. Among these are anti-auxin effects (15), synergistic effects (41), and auxin effects (30). These are no doubt associated with the concentration within the plant.

The data given for slash and loblolly can only be interpreted in general terms since there were no replications for these two species. Gibberellic acid was the only compound that appeared to stimulate epicotyl elongation in both. The higher concentrations of all the other compounds appeared to inhibit the elongation of the epicotyls of these two species. The 2,4,6-T proved to be a very powerful compound causing considerable epinasty. These data do not show one response to 2,4,6-T that was very pronounced. The first application of all of these compounds was put on while the seedlings were in the lag period, i.e. after hypocotyl elongation had stopped and before active epicotyl elongation had started. Five days after application there was considerable elongation in the slash and loblolly seedlings treated with the highest concentration of 2,4,6-T. The seedlings elongated little, if any, after this initial spurt (Figure 8). The seedlings in the other treatments started elongating about a week later.

The response with 2,4,6-T is rather surprising. It may have been due

Table IV

EFFECT OF APPLICATIONS OF GROWTH REGULATORS ON EPICOTYL LENGTHS OF PINE SEEDLINGS

Growth-regulator	Amount (ug)	Longleaf[a] (cm)	Slash (cm)	Loblolly (cm)
Control	0	0.61	5.9	4.7
Kinetin (K)	0.01[b]	0.70	8.7	5.1
	0.1	0.86	6.3	5.0
	1	0.64	3.3	3.9
Gibberellic acid (GA)	1	0.65	6.4	5.1
	10	0.78	8.3	6.1
	100	0.90	10.2	7.6
Indoleacetic acid (IAA)	1	0.62	6.4	5.8
	10	0.53	6.5	3.8
	100	0.59	4.1	3.6
2,4,6-trichlorophenoxyacetic (2,4,6-T)	1	0.51	5.6	5.7
	10	0.57	5.8	5.1
	100	0.89	1.6	1.4
Triiodobenzoic acid (TIBA)	1	0.73	7.8	4.8
	10	0.60	7.7	5.2
	100	1.01	3.2	3.6
Benzotriazole (BT)	1	0.52	9.6	4.9
	10	0.76	7.4	4.8
	100	0.55	6.3	3.5

a. LSD 0.05=0.26 in longleaf only.
b. 0.01, 0.1 and full strength of a saturated ethanol solution of kinetin.

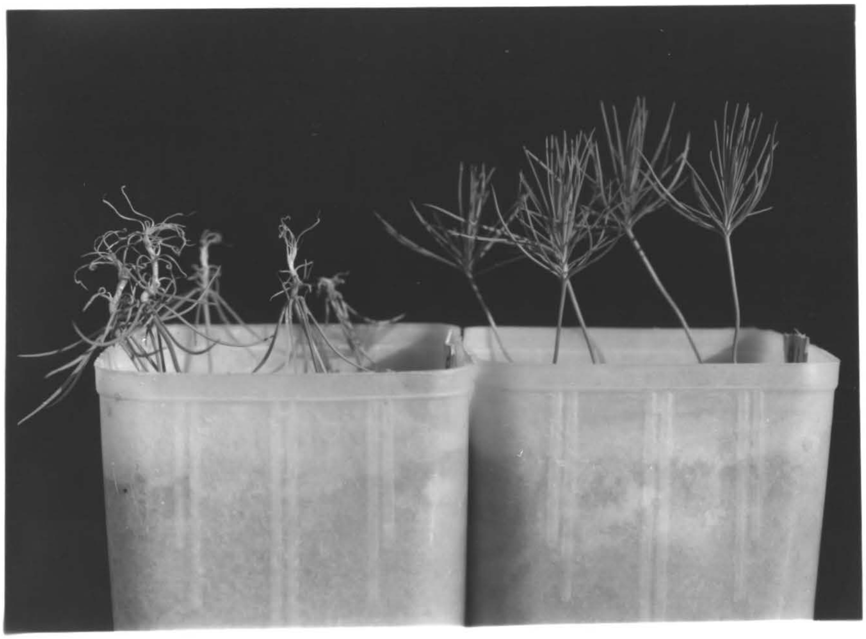


FIGURE 8. EFFECT OF 2,4,6-T ON SLASH PINE SEEDLINGS

Left--treated. Right--control.

to some impurity but that is not likely. Hoffman (19) reports no epinastic response to 2,4,6-T at concentrations up to 1,000 ppm on tomato. This compound warrants further testing on pine.

The loblolly seedlings treated with the two higher concentrations of TIBA all had secondary needles at the time of measurement. None of the other loblolly seedlings produced any secondary needles nor did any of the slash seedlings.

Applications of IAA appeared to have an inhibitory effect upon the epicotyl elongation of slash and loblolly seedlings. The same trend is exhibited in another test (Appendix I, Test 16). In this, IAA again appeared to inhibit the elongation of slash and loblolly epicotyls but had little effect upon Sonderegger or longleaf seedlings (Table V). Kinetin used alone appeared to be inhibitory on loblolly, to have no effect on longleaf and, if anything, stimulatory on slash and Sonderegger. However, when kinetin and IAA were used in combination it appeared to reduce epicotyl elongation in slash, loblolly and Sonderegger. There was little effect on the longleaf with these treatments.

Gibberellic acid appeared to cause some increase in epicotyl length in the slash and longleaf seedlings but not in the loblolly. The difference was not significant in the Sonderegger seedlings.

Since TIBA and GA both appeared to cause some stimulation of longleaf epicotyls they were tested more intensively. In the first of these tests, TIBA and GA were applied alone and in combination to the seedlings at rates of 10 and 100 ug of each per seedling (Appendix I, Test 17). There were two of these tests, one conducted at Durham and one in south Mississippi. In the former all of the seedlings treated with GA and TIBA in combination died. The first evidence of the onset of mortality was that the cotyledons

Table V

EFFECT OF GA, IAA, AND K UPON THE EPICOTYL LENGTH PER SEEDLING OF PINE

Treatment	Slash (cm)	Loblolly (cm)	Sonderegger (cm)	Longleaf (cm)
Untreated control	6.8	2.4	1.9	0.5
Alcohol control	9.6	2.5	1.8	0.6
Indoleacetic acid (IAA)	5.8	1.3	1.8	0.6
Kinetin (K)	7.7	1.0	2.5	0.7
Gibberellic acid (GA)	11.2	2.4	2.3	0.9
IAA + GA	9.1	1.4	2.3	0.7
IAA + K	4.0	0.0	1.0	0.6
GA + K	7.9	0.2	2.0	0.7
GA + K + IAA	5.8	0.4	1.3	0.8

turned a rather bright, brownish-yellow color. The color was quite distinctive and showed up in other tests where the two compounds were used together on seedlings.

There was a significant difference among treatments where there was no mortality. Average epicotyl length per seedling was 4.8 mm for the untreated controls compared to 5.3 mm and 6.6 mm for the TIBA-10 and TIBA-100 treatments respectively. The GA treatments averaged 6.8 mm and 5.5 mm for the 10 and 100 ug rates. The TIBA-100 and GA-10 treatments differed significantly from the untreated controls.

In the test conducted in south Mississippi there was no mortality in the treatments where TIBA and GA were applied together. Although the treatments did not differ significantly in the longleaf, they did tend to confirm that there was some increase in epicotyl length resulting from applications of GA or TIBA. In fact, the combination treatments appeared to be better than either compound alone.

The treatment differences in the slash component of this latter test were not statistically significant. There were the same general trends as in the earlier test. The TIBA tended to reduce epicotyl elongation and GA tended to increase it.

In a similar test where seedlings from natural hybrids as well as longleaf were used (Appendix I, Test 18), GA and TIBA again caused some stimulation of epicotyl growth in longleaf (Table VI). GA alone caused some stimulation but not as much as TIBA alone. The combination treatment, TIBA plus GA, produced significantly more growth than any other.

GA had no effect upon epicotyl growth in hybrid seedlings which had an average hypocotyl length of 1 cm. The higher rate of TIBA significantly inhibited epicotyl growth. The combination of TIBA and GA increased the

Table VI

EFFECT OF TIBA AND GA ON EPICOTYL GROWTH OF SONDEREGGER AND LONGLEAF SEEDLINGS

Treatment	Sonderegger		Longleaf
	Long hypocotyl[a] (mm)	Short hypocotyl[b] (mm)	
Control	9.0	5.1	2.5
GA-5	12.5	4.1	2.9*
GA-50	9.3	4.7	2.8*
TIBA-5	8.3	4.5	3.5**
TIBA-50	9.4	3.6*	3.3**
TIBA-5 + GA-5	11.4	6.9*	3.8**

* Differs from the control at the 0.05 level.
** Differs from the control at the 0.01 level.
a. Average length 4.6 cm.
b. Average length 1.0 cm.

epicotyl growth significantly.

TIBA did not have any effect on epicotyl elongation of hybrid seedlings whose hypocotyls averaged 4.6 cm. The low rate of GA caused some increase but the seedlings treated with the high rate were no different than the checks. The combination of TIBA and GA produced a little effect but it was not statistically significant.

In another test involving TIBA and GA, the TIBA was sprayed on the seedlings in concentrations of 20, 200, and 2,000 ppm (Appendix I, Test 19) and the GA was applied at the rate of 100 ug per seedling with a micro-pipette. Again, there was considerable seedling mortality where both compounds were applied and also where GA alone and the highest concentration of TIBA alone were used. The seedlings in the entire test (including the untreated controls) appeared to be in poor vigor and there were no significant differences in epicotyl length among the treatments which had a full complement of living seedlings.

TIBA was sprayed on seedlings weekly in another test (Appendix I, Test 20) at the same concentrations as used above. There was no mortality associated with treatment in this test although the seedlings sprayed with 2,000 ppm TIBA were not vigorous. There were no significant differences attributable to treatment.

TIBA was applied at the rate of 0, 10, 100, 250, 500 and 1,000 ug per seedling (Appendix I, Test 21) to find the optimum concentrations. The highest concentration caused considerable mortality. There was no mortality with the lower concentrations however. The epicotyl lengths averaged: control, 4.8 mm; 10 ug per seedling, 5.0 mm; 100 ug, 7.9 mm; 250 ug, 7.1 mm; and 500 ug, 4.4 mm. The 100 and 250 ug treatments were significantly different from the untreated control and the 500 ug treatment.

Since TIBA reportedly has auxin antagonistic properties it was decided to test the effect of several other compounds (Appendix I, Test 22). Trans-cinnamic acid at rates of 10, 100 and 500 ug per seedling had no effect upon epicotyl elongation in longleaf. Two,4,6-T at 100 ug per seedling proved to be injurious but at 50 ug per seedling it caused some stimulation (7.9 mm compared to 4.8 mm for the untreated controls). The epicotyls of seedlings treated with 10 ug applications were not significantly longer than those of the untreated controls. Ten and 50 percent applications of DCA killed the terminal meristem regions of the seedlings and the epicotyl length of those treated with one percent applications did not differ from the untreated controls. Epicotyls of seedlings treated with applications of 10, 100 and 500 ug of TIBA averaged 5.0, 7.1 and 5.1 mm respectively. The TIBA-100 treatment was again significantly different from the untreated control.

Indole and coumarin, two compounds with reported synergistic properties (23), were applied to longleaf seedlings in three applications of 10, 100 and 500 ug per application (Appendix I, Test 23). Indole at 500 ug per seedling caused death to the tissue at the point of application, the base of the primary needles. There were no significant differences among treatments in epicotyl length at the other concentrations.

Two replications of slash seedlings were given the same treatments. Indole at the highest rate killed the tip of the epicotyl and thus caused a significant reduction in epicotyl length. At 10 and 100 ug per seedling there was no effect on epicotyl length. Increasing the concentration of coumarin reduced the growth of the epicotyl. Epicotyl lengths for the 10, 100 and 500 ug treatments were 6.1, 5.4 and 3.9 cm respectively. The 10 ug treatment may have stimulated elongation somewhat as this approached a significant increase over the untreated controls. The highest rate was definitely inhibitory. A highly significant increase in epicotyl length was obtained when GA was applied along with the 100 ug applications of either indole or coumarin (8.8 and 8.6 cm for the two GA treatments compared to 5.5 cm for the untreated control). Gibberellic acid was not used alone in this test but in comparing it with another test which was conducted simultaneously it appears that either coumarin or indole may have reduced elongation somewhat as the epicotyl length for the GA-100 treatment in this test was 9.6 cm.

Indole carboxcylic acid was applied four times in applications of 1, 10 and 100 ug per seedling (Appendix I, Test 24). There was no visible effect of these treatments on the needles nor were there differences among treatments in epicotyl length.

Spraying longleaf and loblolly seedlings with glucose or sucrose solutions (Appendix I, Test 25) at concentrations of 5 and 15 percent had no effect upon their epicotyl growth. No growth promotion was noted, but a slight, though statistically insignificant, inhibition of the epicotyl elongation appeared in loblolly seedlings sprayed with alcoholic solutions.

It may be concluded from the foregoing tests that there is a definite possibility of finding a treatment which will stimulate longleaf seedlings in the primary needle stage into making active elongation. The consistent increases in epicotyl length obtained with TIBA and GA were admittedly small but they did show that the epicotyl length can be increased. It may be possible to stimulate the seedlings into making height growth using the compounds employed in these tests once the proper concentrations and techniques are discovered. There are still many other compounds which deserve trial and may prove to be even more effective than the ones used here.

Amino Acid Chromatography

The first amino acid extracts were made of de-needled stem tips of longleaf and slash pine seedlings which had been growing in a sand bench in the greenhouse at Duke University. The entire epicotyl of the longleaf seedling was used. Secondary needles were just becoming visible to the naked eye. The slash seedlings had from 2 to 3 cm of epicotyl length but extracts were made of only the apical 5 mm. The seedlings were growing side by side in the same bench.

The striking thing about these chromatograms of extracts of longleaf stem tips was the tremendous amount of arginine that appeared when the papers were sprayed. When the extract, equivalent to 20 mg green weight of original tissue, was chromatographed the volume of arginine was so large that many of the other amino acids were either covered or pulled out of position by it. The same concentration of slash extract gave excellent chromatograms. It was necessary to use about 1/40 of the original longleaf extract to get good chromatograms. It was then possible to verify that this amino acid was arginine by Rf and co-chromatography. Additional proof was obtained when it was found sensitive to the a-napthol:hypochlorite reagent (Block _et_. _al_. 6). The same results were obtained from duplicate extracts from this same lot of longleaf seedlings.

Extracts from the tips of elongating terminal buds of longleaf saplings did not contain arginine in quantities large enough to detect when an extract equivalent to 20 mg of original fresh weight was chromatographed. Only aspartic acid, serine, glutamic acid, glutamine, L-alanine, proline and methionine appeared. There was no difference in the slash extracts from those discussed later.

There was little change in the number of free amino acids found in the stem tips of pine seedlings at different stages of development,* nor was there much qualitative difference between species (Table VII). The amino acids were ranked as to their relative concentration on the chromatograms. This was judged by eye using color intensity and size of spot as criteria.

Arginine and glutamic acid were found in the highest concentrations in longleaf and slash embryos. Other amino acids identified in both were L-alanine, aspartic acid, serine, -aminobutyric and one which had a high Rf in both solvents, possibly one of the leucines. Longleaf embryos contained glutamine which was not detected in the slash and slash embryos contained glycine which was not detected in the longleaf.

Arginine, glutamine, asparagine and glutamic acid were found in the highest concentration in the stem tip extracts at all stages of development for all three species. Serine, aspartic acid and -aminobutyric acid were also present in all extracts. Methionine was present in all longleaf stem tip extracts. Methionine was also present in all the five and 12-day-old slash tip extracts but was not detected in the slash epicotyls after elongation took place (which was between the 12th and 19th day). It was detected in only one loblolly extract, from 43-day-old seedlings. Glycine was present in all slash and loblolly stem tip extracts but was not detected in longleaf.

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* Extracts were made of the hypocotyl of longleaf embryos and entire slash embryos from seed which had been in contact with moist paper for three days. Extracts were also made of the epicotyls of germinating slash and longleaf, 5-day-old seedlings, and weekly thereafter for four weeks with the slash and five weeks with the longleaf seedlings. Weekly extracts were made of loblolly epicotyls for five weeks starting when the seedlings were 14 days old.

Table VII

RELATIVE AMOUNTS[a] OF AMINO ACIDS FOUND IN THE EPICOTYLS OF PINE SEEDLINGS BEFORE AND DURING ACTIVE EPICOTYL ELONGATION

Amino acid	Longleaf[b]		Slash		Loblolly	
	Before	During	Before	During	Before	During
Arginine	1	1	1	1	1	1
Glutamine	2	2	2	2	2	2
Asparagine	3	3	3	3	3	3
Glutamic acid	4	4	4	4	5	4
Serine	5	5	5	6	4	5
-Aminobutyric acid	6	6	9	5	6	7
Aspartic acid	7	8	6	7	9	9
L-alanine	8	7	7		7	6
Methionine	9	10	10			
Leucine	10				13	
Glycine			8	10	8	8
B-alanine					11	10
Tyrosine				9	10	11
Cysteic acid		9		8	12	12

a. Ranked according to concentration on the chromatogram as visually estimated by size of spot and color intensity.

b. In longleaf, before and after appearance of secondary needles.

Thus it appears that there is little difference qualitatively between the free amino acid content of the stem tips of young longleaf, slash and loblolly seedlings. The only amino acid not found in the longleaf that was present in all of the others was glycine. This may have been because the longleaf extract was not applied in heavy enough concentrations.

A rough quantitative measure of the amino acids present can be obtained from the amount of extract applied to the paper. For slash and loblolly extracts, a properly loaded chromatogram was obtained with the extract from 20 mg of tissue. With longleaf, however, the extract from only 5 mg of tissue was required. Thus the longleaf stem tips contained about four times the amount of free amino acids as the slash and loblolly stem tips.

Just what this means in terms of growth is difficult to say. The relatively larger quantity of free amino acids in the stem tips of longleaf pine could be a result of the epicotyl's failure to elongate. It is conceivable that there is some adaptive enzyme system lacking that is necessary for the incorporation of these amino acids into protein before the epicotyl can elongate. An alternative explanation is that the greater quantity of free amino acids in longleaf is simply a reflection of the much greater needle volume. This latter explanation might also explain the reason why there was no appreciable change in the amino acid pool in the stem tips of loblolly and slash pine before and during epicotyl elongation. The amino acid pool could be replenished by the leaves and roots as fast as the acids were being utilized in protein synthesis.

The build-up of arginine found in the seedlings grown in sand under greenhouse conditions may be the result of a mineral deficiency to which longleaf was more sensitive than slash. Coleman (10) observed a build-up in arginine in sulfur-deficient plants.

Chromatograms of the loblolly, slash and longleaf pine stem tip extracts, using a volume of extract equivalent to the same amount of the original tissue for all three species, were also sprayed to detect the sugars. Glucose, fructose and sucrose were present in all extracts in about the same amounts.

Radioactive Feeding Experiments

The tips of two primary needles of young slash and longleaf seedlings were placed in a aqueous solution of D-glucose-U-C^{14} for 24 hours. The seedlings were then divided into five parts: primary needles, cotyledons, epicotyl, hypocotyl and roots. These parts were dried and a radioautogram was then made of them. From the autoradiograms it was clear that there was translocation of labelled material from the two treated primary needles into the other primary needles, roots and epicotyls of both species. There was a suggestion of activity in the cotyledons but it was not strong. No activity was visible in the hypocotyls of either species. The primary needles contained most of the activity in both species. This test showed that there is translocation throughout the seedling when certain labelled compounds are applied to the needles.

There was little difference in labelled products produced by stem tips of slash and longleaf pine which were fed labelled glucose or succinic acid. The distribution of the activity among the metabolites is given in Tables VIII and IX and the location of these compounds on the chromatograms in Appendix II.

Too much importance must not be attached to the quantitative data of Tables VIII and IX since there was only one replication. General similarity in labelling of compounds by the two species with time does give support, however, to the quantitative differences noted. The time series was, in a sense, replication.

Table VIII

DISTRIBUTION OF ACTIVITY RESULTING FROM THE FEEDING OF LABELLED GLUCOSE TO THE STEM TIPS OF SLASH AND LONGLEAF PINE[a]

Compound[b]	Slash Hrs. of feeding 6	 12	 24	Longleaf Hrs. of feeding 6	 12	 24
1. Succinic acid	0.8	1.0	1.2	0.8	0.6	1.2
2. Malic acid	5.8	7.0	8.6	5.8	6.0	9.0
3. Citric acid	0.6	1.6	1.2	0.3	T[c]	5.5
4. Asparatic acid	0.0	0.7	0.0	0.3	T	T
5. Glutamic acid	5.8	8.2	4.9	4.0	2.1	13.1
6. Asparagine	0.0	0.0	0.0	0.0	0.0	0.0
7. Glutamine	1.7	4.0	5.5	4.5	6.0	4.0
8. Pyrollidone-COOH	0.0	0.0	0.0	0.0	0.0	0.0
9. L-Alanine	43.1	14.2	10.4	44.2	42.2	19.0
10. aminobutyric acid	3.9	6.3	15.3	1.3	7.5	12.1
11. Proline	0.0	0.0	0.0	0.0	0.0	0.0
12. Glucose	*	*	*	*	*	*
13. Sucrose	24.6	38.1	30.1	28.7	19.6	21.0
14. Fructose	0.0	2.3	4.3	0.0	0.0	0.0
15. Unknown	0.0	0.0	0.0	0.8	0.6	0.7
16. Unknown	0.0	0.0	0.0	0.0	0.0	0.0
17. Unknown	0.0	0.0	0.0	0.0	0.0	0.0
18. Unknown	0.0	0.0	0.0	0.0	0.0	0.0
19. Unknown	0.0	0.0	1.8	0.0	0.0	0.0
20. Unknown	0.0	0.5	1.9	3.4	12.0	5.5
21. Unknown	0.0	0.0	0.0	0.0	0.0	0.0
22. Unknown	0.0	0.0	0.0	0.0	0.0	0.0
23. Unknown	2.7	4.9	6.8	0.0	0.0	1.4
24. Phosphorylated compounds	10.4	11.2	8.0	6.0	3.3	7.4

a. Percent of total activity.
b. Numbers refer to location on chromatogram—Appendix II.
c. Trace—activity too low to count.
* Activity of substrate not included in total.

Table IX

DISTRIBUTION OF ACTIVITY RESULTING FROM THE FEEDING OF LABELLED SUCCINATE TO THE STEM TIPS OF SLASH AND LONGLEAF PINE[a]

Compound[b]	Slash Hrs. of feeding 6	12	24	Longleaf Hrs. of feeding 6	12	24
1. Succinic acid	*	*	*	*	*	*
2. Malic acid	54.3	49.4	28.5	39.6	47.9	60.1
3. Citric acid	1.4	2.4	1.2	1.5	2.3	1.7
4. Aspartic acid	4.4	1.7	1.2	5.1	2.4	3.8
5. Glutamic acid	13.5	12.9	8.7	12.8	13.3	4.8
6. Asparagine	0.0	0.0	0.0	1.0	0.6	0.0
7. Glutamine	4.6	5.6	9.8	23.2	17.5	4.8
8. Pyrollidone-COOH	0.3	0.0	0.0	0.0	0.0	1.0
9. L-Alanine	10.3	9.1	7.2	8.7	7.3	6.2
10. aminobutyric acid	5.4	13.4	32.9	5.8	8.5	9.6
11. Proline	0.3	0.3	0.6	0.6	0.2	0.0
12. Glucose	0.0	0.0	0.0	0.0	0.0	0.0
13. Sucrose	0.0	0.0	0.0	0.0	0.0	0.0
14. Fructose	0.0	0.0	0.0	0.0	0.0	0.0
15. Unknown	0.0	0.0	0.0	0.0	0.0	0.0
16. Unknown	1.0	1.1	1.7	1.3	0.1	2.1
17. Unknown	0.0	0.5	0.0	0.0	0.0	3.4
18. Unknown	0.1	0.5	0.8	0.0	0.0	1.4
19. Unknown	0.0	0.0	0.0	0.0	0.0	1.0
20. Unknown	0.0	0.0	0.0	0.0	0.0	0.0
21. Unknown	3.9	1.5	1.7	0.0	0.0	0.0
22. Unknown	0.6	1.3	4.2	0.0	0.0	0.0
23. Unknown	0.0	0.0	0.0	0.0	0.0	0.0
24. Phosphorylated compounds	0.0	0.3	0.5	0.3	0.0	0.0

a. Percent of total activity.
b. Numbers refer to location on chromatogram--Appendix II.
* Activity of substrate not included in total.

The phosphorylated compounds, found in the glucose feeding especially, are probably those necessary in normal glycolysis. With the system of chromatography employed it was not possible to identify them.

The fact that activity was detected in three members of the citric acid cycle: succinate, malate, citrate, when glucose was used as the substrate and that labelled malate and citrate were found with succinate as the substrate, is good evidence that the citric acid cycle is operating in pine. The presence of labelled glutamic and aspartic acid is also evidence of functioning of the citric cycle since these amino acids are formed by transaminations involving -ketoglutaric acid and oxalacetic acid which are both members of the cycle.

Pyruvic 2-C^{14} was fed to stem tips in the same way as was glucose and succinate but for some cause the total activity extracted was very low. The little activity that showed on the chromatograms was in malic, citric, -aminobutyric and glutamic acids and glutamine.

There was nothing in these data which indicated that the dwarf-growth habit in longleaf pine is the result of some abnormality in the utilization of glucose, succinic acid or pyruvic acid.

Auxin Chromatography

Only the ether-soluble *acidic* substances were examined in this work. This is admittedly only looking at part of the substances that have been reported as having growth regulating properties, but adequate techniques will have to be worked out before the ether-soluble *neutral* substances can be investigated in pines. The ether-soluble neutral fraction contains a considerable amount of material (presumably fats, oils and gums) that interfere with chromatography. There were also substances always present in the acidic fraction that caused interference in chromatography.

The most suitable solvent and consequently the one most frequently used, was the isopropanol:ammonia:water solvent. Other solvents were also very useful in certain phases of the work.

The best separations were obtained using two-dimension chromatography where isobutanol:methanol:water (8:1:1) was used in the first direction and isopropanol:ammonia:water was used in the second direction. Figure 9 shows the positions of the ether-soluble acidic compounds consistently present in pine. These were located by viewing under ultra-violet light or by the use of the Erlich or ferric:perchloric spray reagents. Other compounds were present in certain extracts but since they did not appear on all replications they are not included in Table X. Since the compounds extracted are all unknowns they are identified by numbers.

Compounds 1 and 2 did not show any color reaction with either of the two spray reagents. It is entirely possible that they were not present in high enough concentrations to be detected. Compound 2 does show up quite plainly under ultra-violet light and should be present in high enough concentration to produce a color reaction however and conceivably these are not indole compounds.

When extracts were chromatographed in one direction with isopropanol:ammonia:water as the solvent, the rate of travel of Compound 2 was retarded and it traveled along with Compound 6.

Compound 1 is present in a rather low concentration in comparison to some of the other compounds. It was detected in the extracts of the buds from the longleaf, slash and loblolly saplings and in epicotyls of slash seedlings but was not detected in extracts of the epicotyls of non-elongating longleaf pine seedlings. It could not be detected even when very

FIGURE 9. LOCATION OF ETHER-SOLUBLE ACIDIC SUBSTANCES EXTRACTED FROM THE STEM TIPS OF SLASH, LOBLOLLY AND LONGLEAF PINE

Numbers refer to numbers in Table X. IAA used as reference compound. It was not detected in extracts.

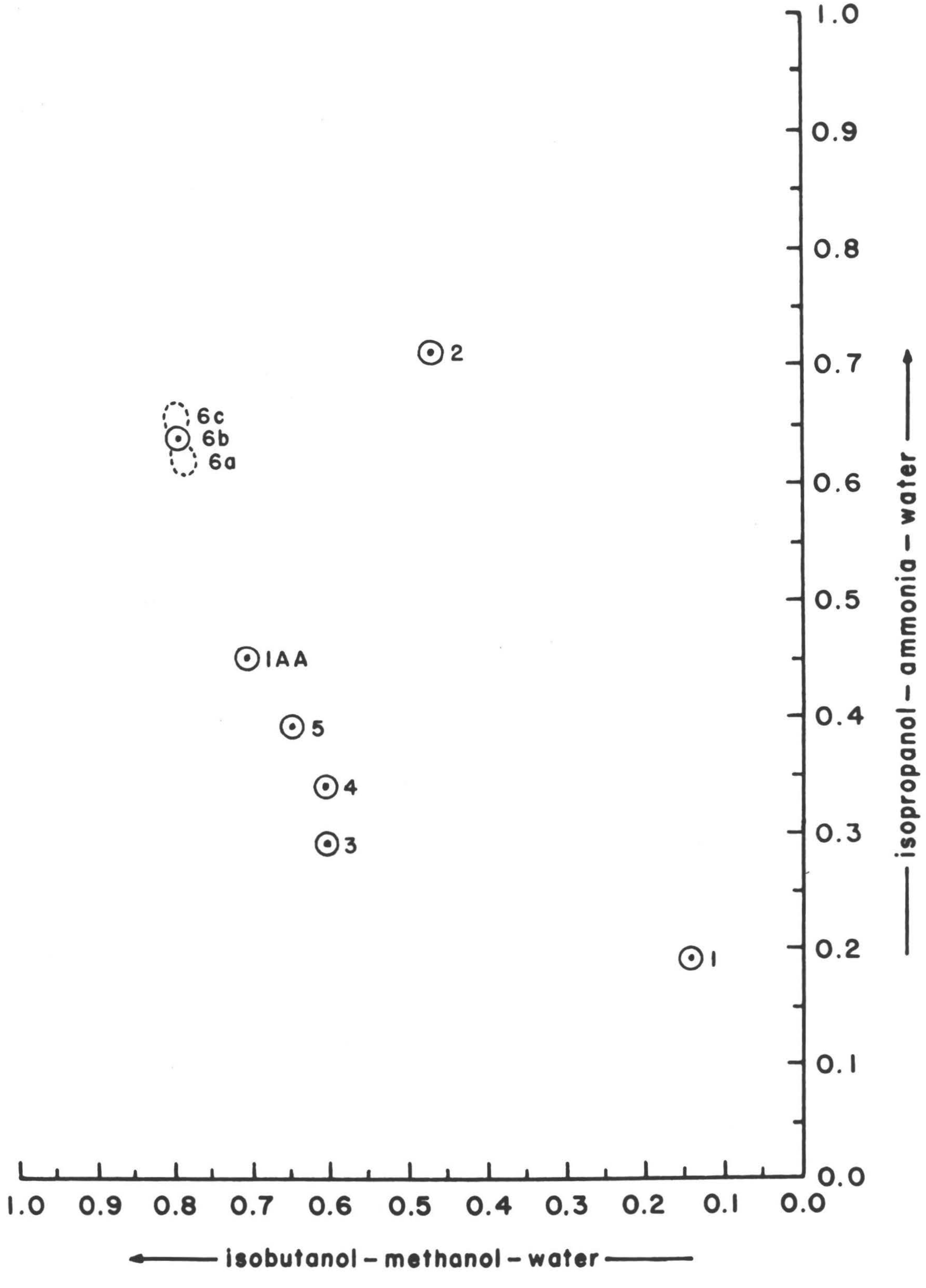

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
isopropanol – ammonia – water
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
isobutanol – methanol – water
2
6c
6b
6a
IAA
5
4
3
1

Table X

COLOR REACTIONS OF ETHER-SOLUBLE ACIDIC SUBSTANCES EXTRACTED FROM THE STEM TIPS OF SLASH, LOBLOLLY AND LONGLEAF PINE

Compound[a]	Ultra-violet fluorescence	Erlich color	Ferric:perchloric color
1.	Blue-purple	None	None
2.	Purple	None	None
3.	Purple-blue	Blue	Blue
4.	Powder blue	Blue	Blue
5.	Dark purple	Deep purple	Deep purple
6A.	Yellow	Yellow-brown	Yellow-brown
6B.	None	Pink-purple	Pink-purple
6C.	None	Yellow-brown	Yellow-brown
IAA.[b]	Lavender	Pink-purple	Red-purple

a. Numbers refer to the numbers in Figure 9.
b. Reference compound. Not identified in extracts.

high concentrations of the extract were used.

Compounds 3 and 4 travel as one spot in the isobutanol:methanol:water solvent. They separate out into two compounds in isopropanol:ammonia:water. They may be due to some complexing with the ammonia but when they were run in two directions in the latter solvent only two spots were obtained rather than the three or four that would be expected if there were some complexing or breakdown occurring. This technique is similar to that used by Schwarz and Bitancourt (36) in their investigation of indole pyruvic acid.

It was necessary to use fairly high concentrations of extract to be able to detect these compounds with the spray reagents. They were easily detected with ultra-violet even at low concentrations, since they fluoresced very intensely.

Compounds 3 and 4 were autoclaved with an acid and a base then extracted with ether and chromatographed. Both compounds could be detected by their fluorescence and chromogenic reactions after two hours of autoclaving in 2N KOH but were not detected after one hour of autoclaving in five percent HCl. Indoleacetic acid is also destroyed by hot acid but is quite stable in a hot base.

These compounds were chromatographed and eluted from the paper. Their ultra-violet absorption was then checked in a spectrophotometer. Both compounds gave a peak absorption at 285 mu as did indoleacetic acid. The curves were not very distinct, presumably due to combination of low concentration and interfering substances but the fine details were lost so it was not possible to differentiate between two compounds.

The two compounds were present in larger quantities in the elongating buds of slash, loblolly and longleaf saplings than in the extracts of the epicotyls from non-elongating longleaf seedlings and the elongating epicotyls of slash seedlings.

Compound 5, in low concentration, was detected on chromatograms of extracts of elongating buds of slash, loblolly and longleaf saplings as well as in the extracts of the epicotyls of non-elongating longleaf seedlings. This compound chromatographed nearer to IAA than any other compound detected. Its ultra-violet fluorescence and color reactions, however, were not like those obtained with IAA (Table X).

The compounds in the 6 area were difficult to evaluate. The compound labelled 6A fluoresces while 6B does not. Six C was yellow-brown in color after spraying with Erlich and Salkowski reagents. In slash and loblolly extracts, Compound 6B produced a purple color when sprayed with either of the two spray reagents while in longleaf extracts, a pink color was produced. This may be because they are different compounds or it may be an interaction produced by different concentrations of over-lapping compounds or possibly a dilution effect. In one-dimension chromatography with isoproponal:ammonia:water solvent the rate of travel of Compound 2 is retarded so it also occurs in this zone. Some compounds in the 6 area were not destroyed after two hours of autoclaving in 2N KOH or 10 percent HCl. Erlich color reacting compounds appeared in their normal positions after they were chromatographed in one direction.

Compound 6B from longleaf was purified to a considerable degree by chromatographing, eluting, and re-chromatographing through a series of solvents (isopropanol:ammonia:water; isobutanol:methanol:water; acetic acid: water and isopropanol:ammonia:water again). This provided the information that this compound does not fluoresce. Purification procedures were not employed on the compounds in the 6 area for any of the other species.

Since there were differences in reaction, it may be tentatively stated that there are three compounds in the 6 area after two-dimension chromatography (Figure 9). They are so merged that a more positive statement will have to await additional work.

There is one other compound (Compound 7) that warrants discussion. This compound could be detected on one-dimension chromatograms in the isopropanol:ammonia:water solvent appearing as the brightest fluorescing spot on the papers which were bio-assayed (see next section). It did not produce a color reaction with the two reagents employed. When chromatograms were run in isopropanol:ammonia:water solvent in the first direction, the compound could be detected, but did not fluoresce after chromatographing in the second direction in isobutanol:methanol:water. However, when the extracts were run in the latter solvent first and then in the isopropanol: ammonia:water, it could not be detected in either direction. It also lost its fluorescense in a day when the papers were stored in air in the dark. It was visible, however, after a portion of the raw extract was autoclaved for two hours in 10-percent HCl and then chromatographed.

This compound is probably the same type of compound as observed by Palmquist (32). He lists a number of woody species that contained substances which fluoresced brilliant yellow when exposed to ammonia vapor. These lost their fluorescence when exposed to HCl vapor but would regain it upon re-exposure to ammonia.

All of these compounds remain unidentified. Their Rf's do not agree with any of the compounds whose Rf's are given in Appendix III.

Bio-Assay of Growth Substances

Straight Growth Tests

Sections of etiolated slash pine hypocotyls proved to be satisfactory test objects. Preliminary testing showed that they responded to IAA in a manner similar to oat coleoptile sections. The growth of slash pine sections was 133, 160, 187 and 160 (expressed as percent of controls) when grown in solutions containing 0.01, 0.1, 1 and 10 ppm IAA.

The sections grew very little when placed in solutions without auxin. Thus the test for inhibitors on the chromatogram strips were not very sensitive. For this reason 0.1 ppm IAA was included in the test solution. Preliminary testing with extract from elongating buds of longleaf saplings showed that the zones of growth promotion on the chromatograms were still readily apparent though not quite as pronounced as where IAA was not used. The zones of inhibition showed up more clearly. Only one area, Rf 0.9 - 1.0, responded differently. There was no promotion without IAA but there was substantial promotion when IAA was included in the test solution. This was probably due to the presence of some compound exerting an additive or synergistic effect.

The conditions under which these tests were conducted were far from satisfactory. Temperatures often dropped to 15°C which is considerably below optimum temperature for slash pine growth. The sections from seedlings grown at the lower temperatures did not respond well and they grew little when the temperature dropped while they were actually in test. Only qualitative comparisons can be made between the histograms presented for the different extracts since most of the tests were conducted at different times. More definitive appraisal will have to await better facilities.

Results of the bio-assays of the extracts with hypocotyl sections are presented in Figures 10 and 11 in which activity is plotted against Rf value. The horizontal line in each diagram represents the level of growth of the controls treated with 0.1 ppm IAA.

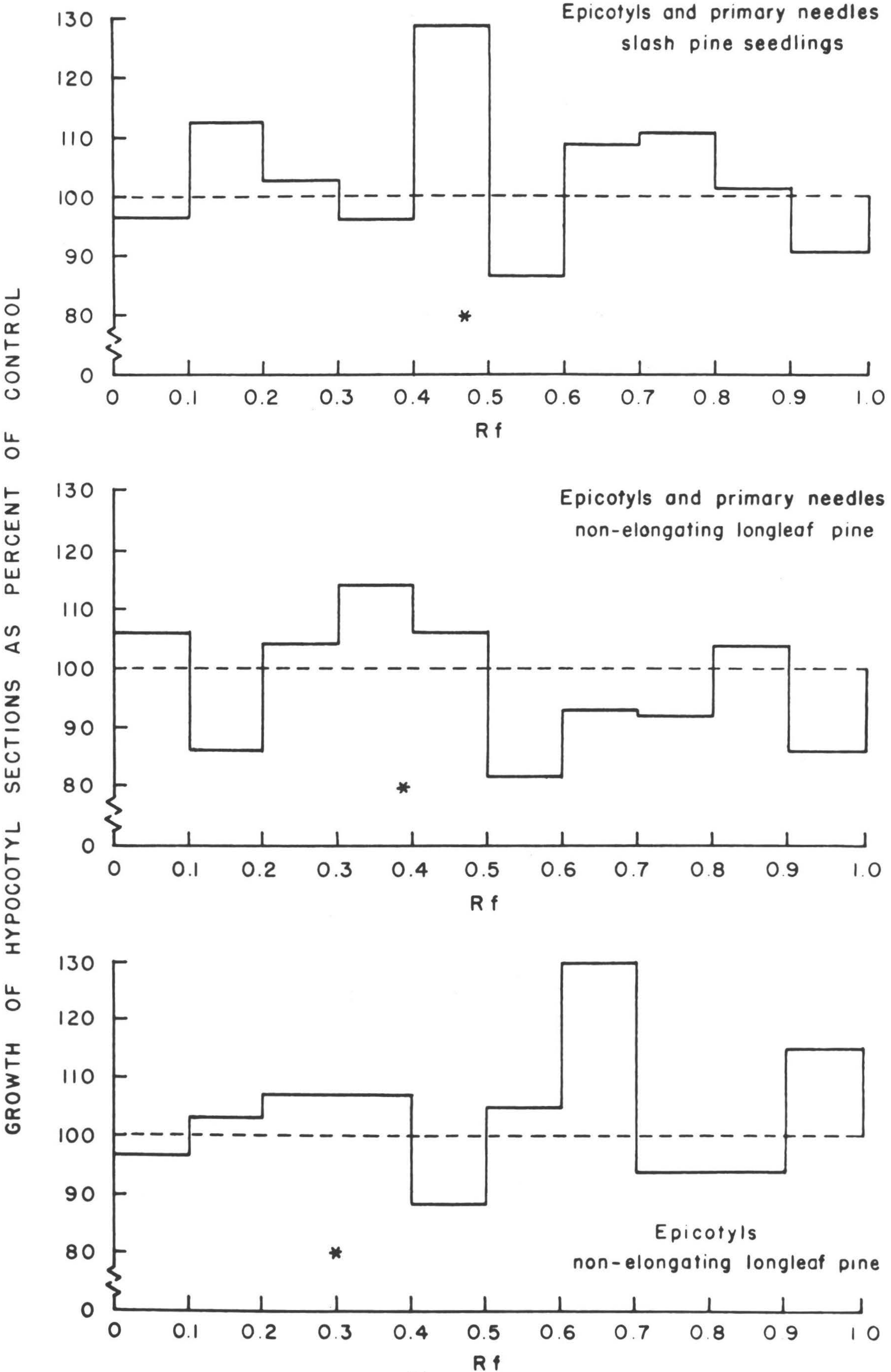
There was one zone of growth promotion that appeared on chromatograms of all extracts. This zone varied from Rf 0.2-0.4 in the extracts of non-elongating longleaf to Rf 0.6-0.7 in the slash sapling extracts. The growth promotion is thought to be due to the same compound in all extracts since its position is very well correlated with the yellow fluorescing spot, Compound 7, discussed earlier.

The position of Compound 7 is marked in Figures 10 and 11 where one can see that it did not travel as far in the chromatograms of non-elongating longleaf extracts as it did in those of elongating buds of slash saplings. Possibly contaminating substances interfered with the rate of travel. Also it should be noted that the chromatograms were run at different times.

While Compound 7 is in the zone where IAA travels in the solvent system used, Compound 7 is not IAA since it was detected in one-dimension chromatography after treating with hot acid but IAA was not. The only other compound detected in this area was Compound 5. But Compound 5 did not prove to be IAA either since it did not chromatograph exactly the same as IAA in two-dimension chromatography nor did it show the same fluorescence or chromogenic reactions. When Compound 5 was cut out of two-dimension chromatograms and tested with hypocotyl sections, without adding IAA to the test solution, it proved to cause growth promotion (95 percent greater elongation than the controls).

FIGURE 10. GROWTH OF PINE HYPOCOTYL SECTIONS ON PINE EXTRACTS

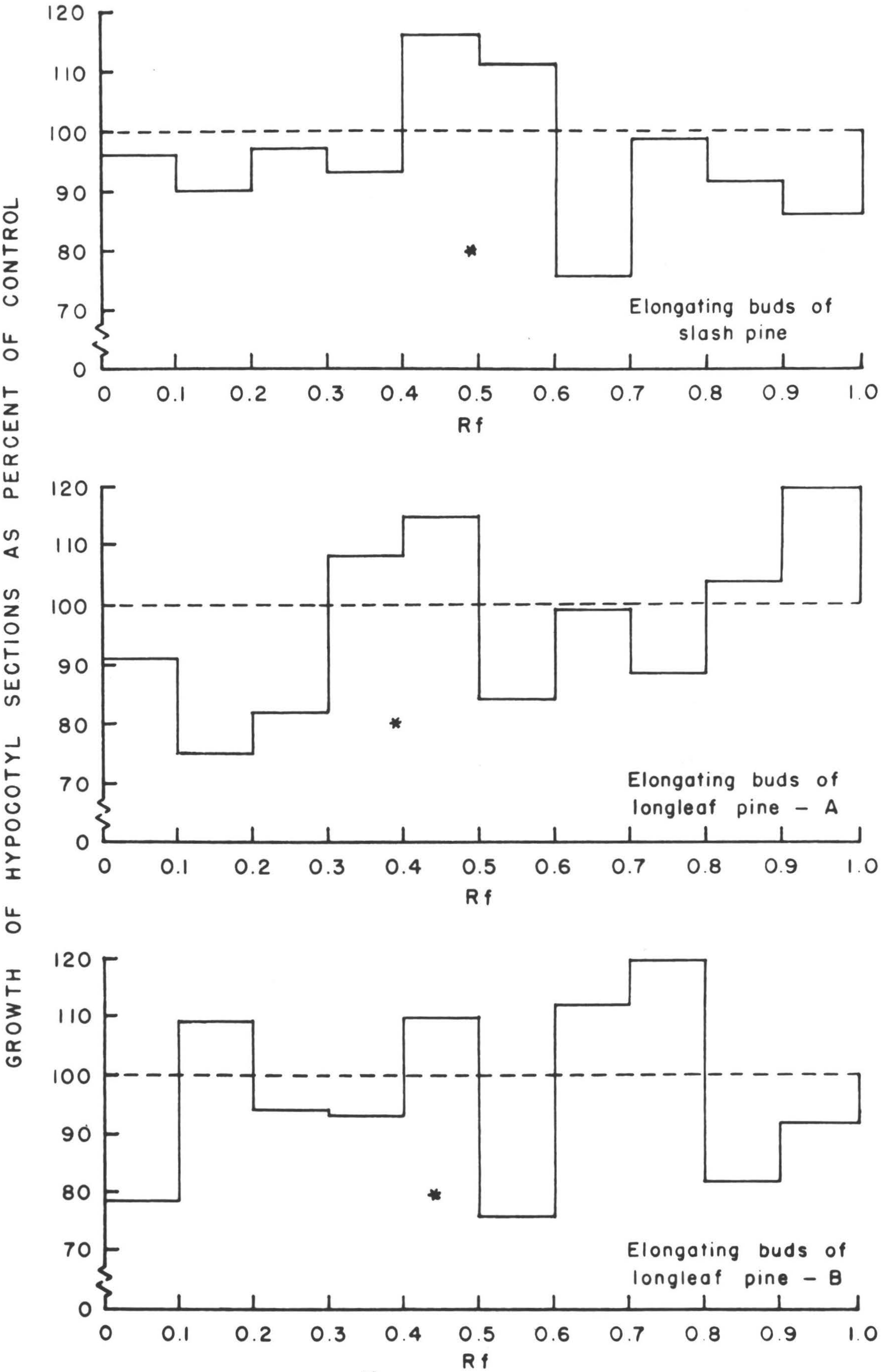
Chromatographed in isopropanol:ammonia:water. Asterisk denotes position of Compound 7 on chromatograms.



Epicotyls and primary needles
slash pine seedlings
Epicotyls and primary needles
non-elongating longleaf pine
Epicotyls
non-elongating longleaf pine
GROWTH OF HYPOCOTYL SECTIONS AS PERCENT OF CONTROL
130
120
110
100
90
80
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Rf
*

FIGURE 11. GROWTH OF PINE HYPOCOTYL SECTIONS ON PINE EXTRACTS

Chromatographed in isopropanol:ammonia:water. Asterisk denotes position of Compound 7 on chromatograms.


GROWTH OF HYPOCOTYL SECTIONS AS PERCENT OF CONTROL
120
110
100
90
80
70
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Rf
Elongating buds of slash pine
Elongating buds of longleaf pine – A
Elongating buds of longleaf pine – B
*

Some of the growth promotion obtained in the area of Rf 0.2-0.4 was due to Compound 3. This compound caused some stimulation in growth of both *Avena* sections and slash hypocotyl sections when it was cut from two-dimension chromatograms. Compound 4 did not differ from the controls in these tests. Compounds 3 and 4 had the same Rf when chromatographed in isobutanol:methanol:water. The blue fluorescent spot, which corresponded to the position of these two compounds, was cut out and tested with hypocotyl sections without adding IAA to the test solution. Increases of 50 percent in growth over untreated controls were obtained.

There was a zone of promotion with an Rf of 0.7-0.9 depending upon the extract. This zone was not evident in extracts of non-elongating longleaf seedlings with primary needles, elongating buds of slash saplings, or extract "A" of elongating buds of longleaf saplings. This is thought to be due to the masking of the compound by an inhibitor. In the extract "B" of elongating buds of longleaf saplings the zone of promotion was quite pronounced. This extract was partially purified by chromatographing it with acetic acid:water solvent until all of the soluble substances had moved off the paper and then extrating the eluate. When it was re-chromatographed in isopropanol:ammonia:water this zone of promotion was readily apparent.

This area (Rf 0.7-0.9) is hard to evaluate since it is in the area in which there is a number of compounds occuring in one-dimension chromatography. The compounds of Area 6 in Figure 9 and Compound 2 all occur in this region. The zone of inhibition could be caused by an interaction between two or more of the compounds since there is considerable over-lap among them.

The purified longleaf extract was run in two-dimensions and the compounds cut out and tested. Compound 2 did not differ significantly from the control. Some of the spots cut from Area 6 showed definite growth promotion while others showed definite inhibition. This is thought to be the result of not being able to locate the compounds precisely enough on the chromatograms. The different results were due to differences in concentration of the various substances among the spots cut out. This area will require more work and probably a more suitable solvent system before the compounds can be isolated and their individual effects ascertained.

There is one other zone of growth promotion that occurs on these chromatograms. This is in the area of Rf 0.1-0.2 and was detected only in the extracts of the slash seedlings and purified extract of elongating buds of longleaf. This zone of promotion is in the area where Compound 1 occurs with this solvent. Marked inhibition occurred in this zone with the extract of the longleaf epicotyls plus primary needles.

Compound 1 was located on two-dimension chromatograms in all extracts except of the non-elongating longleaf seedlings. When it was cut out of two-dimension chromatograms and tested with slash hypocotyl sections a slight growth promotion was noted. Therefore its effect must be masked by inhibiting substances occupying the same area in chromatograms of other extracts of elongating buds. This compound was not detected in extracts of non-elongating longleaf epicotyls by either fluorescence in two-dimension chromatography or by bio-assay in one-dimension chromatography. Thus Compound 1 does not appear to be present in the epicotyls of non-elongating longleaf, or if present, is in much smaller amount than in the other tissues examined or its effects are masked by inhibitors having the same Rf values.

The areas of growth promotion isolated on the chromatograms are fairly well established. The areas of growth inhibition, except for the one located in Rf 0.5-0.7, are not. There seemed to be more variability from extract to extract in the inhibiting substances than in the promotion substances. This is possibly due to interactions between the IAA included in the test solution and compounds in the extract. Another reason is that most of the compounds that were isolated on two-dimension chromatograms proved to be growth promoting and not inhibiting substances. The inhibitors evidently could not be located by ultra-violet or the spray reagents employed. If the growth inhibiting substances could have been checked on two-dimension papers as Compounds 1, 2, 3, 4 and 5 were, it might have been possible to interpret some of the variation obtained in Figures 10 and 11.

Flax Tests.

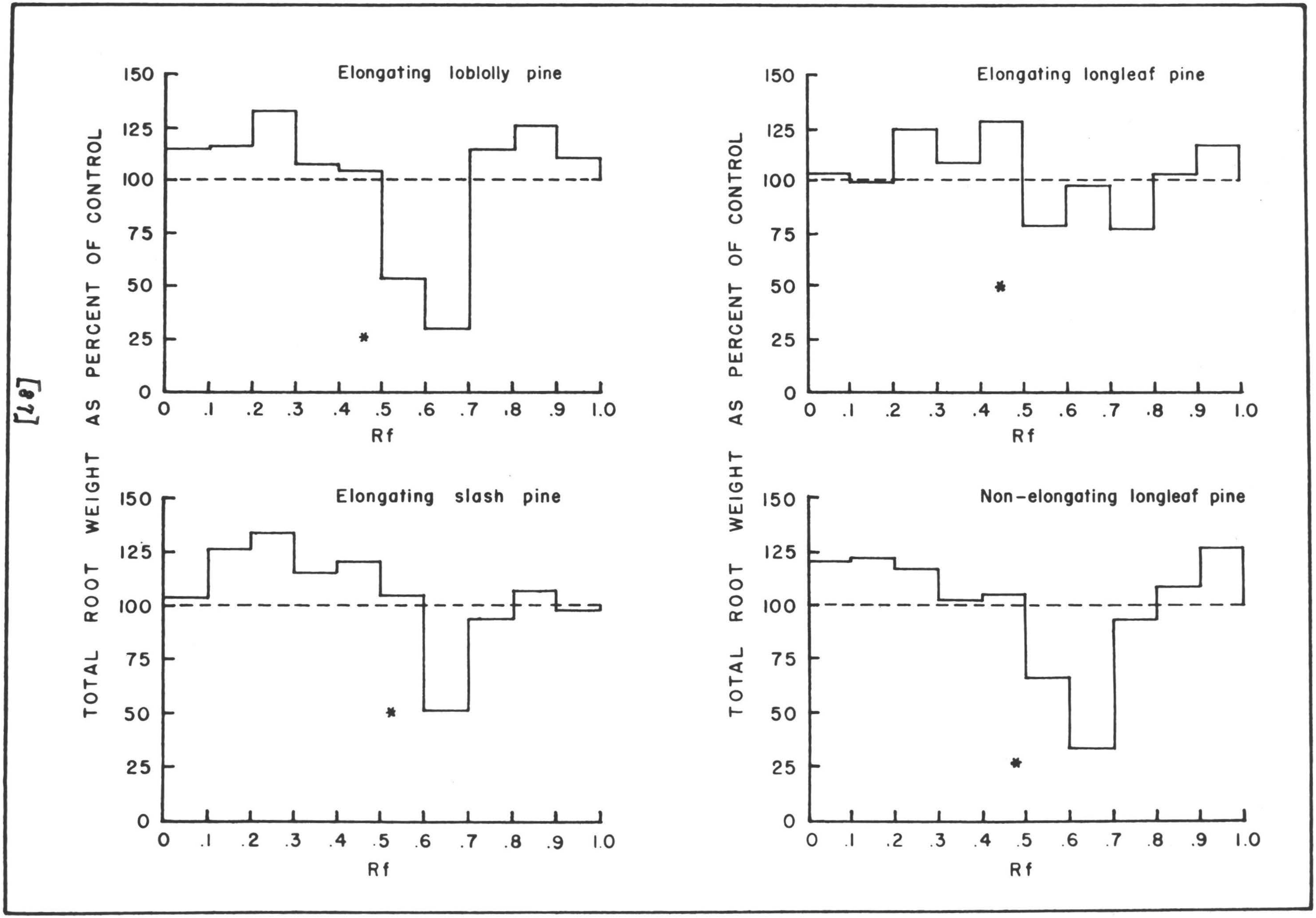
Some of the extracts used in the hypocotyl tests were also assayed by germinating flax seed on the chromatogram sections. After two days the roots were broken off at the seed and weighed. The results were plotted on Figure 12. In preliminary testing it was found that IAA at 10 ppm inhibited root growth of untreated controls by 50 percent; at 0.01 ppm, 13 percent inhibition was obtained. There was a 15 percent increase in growth at 0.001 ppm.

The inhibition of root growth in the vicinity of Rf 0.5-0.7 was the most striking feature of this assay. It corresponds to the same area that was observed in the slash pine hypocotyl test.

In testing the compounds isolated in two-dimension chromatography, it was found that both Compound 2 and the compounds in Area 6 were inhibitory. Only one concentration of extract was used so it is not known whether these compounds are inhibitory at all concentrations. This will need to be in-

FIGURE 12. GROWTH OF FLAX ROOTS ON PINE EXTRACTS

Chromatographed in isopropanol:ammonia:water. Asterisk denotes position of Compound 7 on chromatograms.


Elongating loblolly pine
Elongating longleaf pine
Elongating slash pine
Non-elongating longleaf pine
TOTAL ROOT WEIGHT AS PERCENT OF CONTROL
TOTAL ROOT WEIGHT AS PERCENT OF CONTROL
150
125
100
75
50
25
0
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
Rf
*

vestigated further.

There is one zone of promotion that stands out in the chromatographs of the elongating buds of slash, longleaf and loblolly. This zone is from Rf 0.2-0.4. Compounds 3 and 4 were located in this area and when they were run on two-dimension chromatograms and tested, both proved to promote root growth at the single concentration tried.

The histograms for the various extracts are quite similar. There was nothing particularly different from that of the non-elongating longleaf epicotyls and the others. In fact, it was almost identical to that obtained with the extract of the elongating buds of the loblolly pine. In both longleaf extracts there was a zone of promotion from Rf 0.9-1.0. This is in the same area where a synergistic effect was noted when IAA was included in the straight growth tests with similar longleaf extracts.

DISCUSSION

It is well known that longleaf pine seedlings are different from the other pine species in many respects. Probably many of these differences have their origin in, or are the result of, or are associated with, the processes which cause the dwarf-growth habit.

In slash and loblolly seedlings there is a balance between leaf functions and stem functions. In longleaf there is a preponderance of leaf functions. This is evident even in the embryo where the longleaf cotyledons make up 81 percent of the total weight compared to 48 percent in the slash. The hypocotyl of germinating longleaf seedlings elongates very little but the cotyledons elongate to a considerable length. When slash seedlings are grown in the dark, hypocotyl elongation is increased and leaf growth is retarded but in longleaf, leaf growth is not affected or may even be accelerated. This can be interpreted to mean that leaf growth is controlled by different systems in the two species. Finally, longleaf seedlings produce secondary needles at the time when epicotyl elongation takes place in slash and loblolly seedlings.

It appears that there is an imbalance in food utilization in the longleaf which operates in favor of needle growth at the expense of stem growth. The theory that the cotyledons and the primary and secondary needles compete with the epicotyl for substance (s) necessary for elongation or produce substances which inhibit the growth of the epicotyl, seems plausible. Some support for this theory is found in the fact that longleaf seedlings must be extraordinarily vigorous before beginning height growth. The lower rate of photosynthesis and respiration per unit of needle weight in longleaf as compared to slash and loblolly may tend to prolong the grass stage but it is not a primary cause since the growth habit is expressed in germinating seedlings where there is an abundance of food which can be drawn upon.

The processes which control stem elongation do not seem to be controlled by a single environmental factor. Varying the day length, for example, and subjecting the seedlings to cold did not break the inhibition, and hypocotyls of dark-grown seedlings were only a little longer than those of the light-grown ones.

The radioactive glucose and succinate feeding study showed that there was little difference between the metabolic processes of longleaf and slash at the intermediate metabolism level. Data on the kinds of free amino acids support~~s~~ this contention. Little difference was noted between the free amino acids in the epicotyls of longleaf (except glycine was not detected) and epicotyls of loblolly and slash seedlings. The amino acid pools were, however, considerably larger in longleaf epicotyls than in the other two species. In our present state of knowledge this information is open to several interpretations. In yeast, it seems that amino acids accumulate as a stage in their incorporation into protein (Spiegelman *et.al.* 38) while in staphylococci, Gale (14) finds that protein synthesis is disorganized when there is an accumulation of free amino acids. Often when protein synthesis is occurring under optimal conditions there is no accumulation of free amino acids within the cell. It was determined, however, that there was no drastic change in the amino acid pool in slash and loblolly epicotyls when elongation occurred and protein synthesis must have been taking place. The amino acid pools could have been held at a more or less constant level in the epicotyls by replinishment from the needles and roots or by hydrolysis of protein. Turnover values for amino acids in stem tip proteins require checking.

Since there were very slight differences in intermediary metabolites, qualitative amino acid pool (except possibly glycine), and sugars of the epicotyls of longleaf and slash pine, the assumption that the basic products necessary for growth are present in longleaf can be made. The original premise that the longleaf growth habit is the result of an imbalance between stem and needle growth is logical. This could be under hormonal control and the idea was subjected to experimental testing.

(While) There was no marked elongation in the epicotyls of the longleaf seedlings treated with a variety of growth substances, small but consistent increases were obtained with TIBA and GA. There was some additive effect when the two were used together.

It is not particularly surprising that longleaf seedlings did not make active epicotyl elongation in these tests. Of the compounds applied, only IAA, ICA and gibberellin-like compounds have been found in higher plants but we do not know if they occur in the pines.

The increase in epicotyl growth obtained with gibberellic acid may give insight into the processes involved. It does not seem likely that the dwarf-growth habit of longleaf is due to a genetic blockage in the synthesis of a natural gibberellin factor or there would have been a much greater response in the seedlings.

Although the increase obtained with gibberellic acid was only a few millimeters, on a percentage basis the increase over the untreated controls was nearly as great as that obtained with the other pine species. It appears then that the reduced growth is not due to an absence of some material but rather to some factor in short supply. This factor is probably hormonal in nature since TIBA also produced an increase. It is assumed that the TIBA effect was a synergistic one with one or more of the natural auxins in the seedling. Two other compounds with synergistic action caused some stimulation in longleaf epicotyl growth but the differences were not statistically significant.

If these premises are correct, Compound 1 is a possible cause of failure of elongation in longleaf seedlings. It was not present in quantities large enough to detect on two-dimension chromatograms of extracts of non-elongating longleaf epicotyls and was not detected by bio-assay in one-dimension chromatograms. But the latter is not conclusive evidence that it was not present in detectable quantities because its effect could have been masked by other substances.

Considerably more work should be done on the growth-promoting and inhibiting substances in pine. The present work indicates the general picture but these substances should be checked in more detail. By using only one solvent system some compounds will be masked by others. Too, some of the promotion response might be the result of the additive effect of more than one compound. The quantitative aspects and the effects of concentration should be ascertained.

There seems to be a good correlation between the growth substances found in pine with those found in apple leaves by Luckwill (24). He used a water saturated butanol:ammonia solvent in which IAA and IBA gave Rf values similar to those obtained with the isopropanol:ammonia:water solvent used in the present investigations. He found a growth-promoting substance near the origin (which he calls Auxin 1) that corresponds to the one here obtained at Rf 0.1-0.2. This could be the "accelerator a" of Bennet-Clark and Kefford (5).

It is not likely that this is indole pyruvic acid since this acid was shown to be unstable in the isopropanol:ammonia:water solvent by Bentley et.al. (4).

Luckwill obtained an inhibitor (Inhibitor 1) in the vicinity of Rf 0.35 which he believes to be a toxic substance without biological significance as a growth regulator. The pine extracts did not show any pronounced inhibition in this area. Chromatograms of apple extract had a zone of promotion around Rf 0.45 which he labels "Auxin 2". This is not IAA. A zone of promotion in the same area is found with the pine extracts. At least part of the response in this zone is caused by Compound 5 but Luckwill's Auxin 2 could also be present since it is not detectable by ultraviolet fluorescence nor does it give color reactions with either Erlich or Salkowski-type reagents.

An inhibitor (Inhibitor 2) was present in chromatograms of apple extracts in the same vicinity as found in the pine. Luckwill's Auxin 3 also corresponds to the same area (Rf 0.6-0.7) in which a growth promoting substance was found in the present study.

In resume, it seems that there is a marked similarity between the auxin system in pine and the system found in apple.

SUMMARY

Longleaf pine (*Pinus palustris* Mill.) is an important timber tree in the South, but its growth is discouraged by the fact that the seedlings do not make active height growth until they are three to five years old and mortality is high during this period. A study therefore was planned to investigate some of the physiological processes which might be responsible for the delayed growth.

This study was limited almost entirely to very young seedlings and involved comparison of longleaf with slash and loblolly pine seedlings which make normal epicotyl elongation at a very early age. It was found by various tests and measurements that longleaf seedlings differ from those of the other two species in many ways. The most important difference is the predominance of needle growth over stem growth in longleaf seedlings.

Tests were made to investigate the basic physiological processes of longleaf pine seedlings. Photosynthesis and respiration measurements showed that longleaf seedlings have a lower rate per unit needle weight than either of the other two species. Radioactive techniques were employed in examining the intermediary metabolism of slash and longleaf pine epicotyls. No major differences were detected in the metabolites resulting from incubating the epicotyls in labelled glucose or succinic acid. However, longleaf epicotyls were found to contain a much larger quantity of free amino acids in comparison to the other two species. This was discovered while investigating the amino acids in the epicotyls of the three species at various stages in their normal development. Qualitatively, there was little difference in free amino acids among the species at the stages of development checked.

Attempts were made to stimulate height growth in two ways, by varying the environment, and by applying growth regulating substances to the seedlings. Neither cold treatments or changing the day length was effective in breaking the inhibition.

Emphasis was placed on the tests with the growth regulators since there was a possibility that longleaf differs from other species in the abundance of growth promoting substances. Only two of an assortment of growth regulating substances employed produced any positive response. Gibberellic and triiodobenzoic acids caused a small but consistent increase in epicotyl length.

A survey was made of the ether soluble acidic substances in the epicotyls of pine seedlings and the buds of saplings using paper chromatographic techniques. There are at least four growth-promoting and one growth-inhibiting substances present in pine. One of the growth-promoting substances was not detected in the extracts of non-elongating longleaf pine epicotyls.

Although the problem of retarded epicotyl elongation in longleaf pine seedlings has not been solved, much information was gained in the study which can be profitably used to guide further investigation.

APPENDIXES

APPENDIX I

Description of Tests Made in an Effort to Stimulate Height Growth

Test 1. Vernalization.

Longleaf seeds were vernalized for 12, 6, 4 and 2-week periods before sowing. The seeds were placed on moist filter paper in petri dishes and held at room temperature for three days. The radicle was just visibly emerging from the seed coat at the end of this time.

The seeds were then put in a cloth bag and placed in a desiccator which had water in the well. A small dish of five percent KOH was also placed in the desiccator to prevent a CO_2 build-up. The desiccator was placed in a refrigerator at 5°C. This procedure was repeated for each of the time intervals.

Thirteen days after the 12-week treatment was placed in the refrigerator, the refrigerator warmed up to room temperature for a day. This was the only time this happened.

At the end of the prescribed chilling treatment, the seeds were planted in pots in the greenhouse. Ten seedlings were planted which were thinned to the five largest one month later. The test was installed in a randomized complete block design with four replications.

Test 2. Seedling cold.

Actually there are two tests included here. In the first, the seed for the entire test was sown in pots. Ten seed were sowed per pot and the seedling stand was thinned to five seedlings one week after germination. One, 5, 7 and 8-weeks after germination the seedlings were lifted from four randomly selected pots and the soil washed from the roots. The roots were then wrapped in a wet cloth and the seedlings placed in glassine bag and sealed. The bag was placed in the refrigerator at 5°C until time for transplanting. They were of a two-quart size and only the end was sealed. They contained a fair amount of air. Since the temperature was low it was felt there would be no toxic effects from a CO_2 build-up or oxygen deficiency.

The seedlings were all transplanted to pots in the greenhouse nine weeks after germination. This meant that the seedlings which were one week old prior to subjecting to the cold received eight weeks of cold; those five weeks old received four weeks of cold; those seven weeks old received two weeks of cold; and those that were eight weeks old received one week of cold. One lot of seedlings which had received no cold were lifted and re-potted to serve as an untreated control. Five seedlings were planted per pot. The pots were arranged in a randomized complete block design with three replications.

In the other half of this test the seedlings age at the start of the cold period was held constant and the length of cold treatment was the only variable. The length of the cold treatments were 8, 4, 2, 1 and 0 weeks. To do this seed was sown fourteen days before the start of a cold period. In all cases the radicles of most of the seeds were visible four days after sowing which means that the seedlings were about 10-days-old at the start of the cold treatment.

The seedlings were lifted, the roots wrapped, and the seedlings placed in glassine bags in the refrigerator in the same manner as described in the other half of this test. By starting the seeds at different periods it was possible to complete the treatment periods at the same time and thus transplant into pots simultaneously. It was felt there would be less experimental error with this procedure than if the seedlings were all started at the same time and transplanted at various intervals.

Five seedlings were planted per pot and the pots arranged in a randomized complete block design with three replications.

Test 3. Seed Soaking.

There were eleven treatments in this test:

A. Dry check.
B. Moist cold for 2 weeks.
C. Cold soak for 2 days, room temperature for 1 day, then 2 days cold soak.
D. Cold soak for 2 days, room temperature soak for 2 days, then 2 days cold soak.
E. Cold soak for 6 days, room temperature soak for 1 day, then cold soak for 7 days.
F. Cold soak for 5 days, room temperature soak for 2 days, then cold soak for 7 days.
G. Cold soak for 2 weeks with no change in water.
H. Cold soak for 2 weeks with water changed every 2 days.
I. Cold soaked for 2 days.
J. Soaked at room temperature for 2 days, then cold moist for 2 weeks.
K. Soaked at room temperature for 2 days, then cold soaked for 12 days.

In this test 100 seeds were placed in small plastic containers and 100 cc of tap water used in the soak treatments. In the treatments calling for moist cold the seeds were laid on moist filter paper and the containers sealed. All cold treatments were at 6°C.

Treatments were started at various intervals so that the seed could be planted at the same time. The seeds were planted directly into the pots with 15 seeds each. These were thinned to five seedlings per pot six weeks after germination.

The pots were arranged in a randomized complete block design with three replications.

Test 4. Photoperiod-Cold.

There were eight treatments in this test:

A. Continuous long day.
B. Continuous short day.
C. Short day-long day.
D. Long day-short day.
E. Short day-cold-long day.
F. Short day-cold-short day.
G. Long day-cold-long day.
H. Long day-cold-short day.

Seedlings were germinated under long day conditions (15 hours) and transplanted into pots when they were one week old. There was a total of 24 pots in this test with five seedlings per pot thus making a total of 15 seedlings per treatment. One week after potting they were subjected to the above treatments.

The long-day treatment consisted of giving the seedlings additional light in the morning and afternoon (total day--15 hours) from Mazda lamps hung about five feet above the pots. The short-day treatments were placed in a light-tight box which had a cover that was raised each morning and lowered each evening giving a nine-hour-day.

Six weeks after the treatments had started, the pots of seedlings receiving a period of cold (E, F, G, H) were placed in a cold room at 5°C. They remained there for four weeks after which they were returned to the greenhouse to either long or short-day conditions.

Eight weeks after the treatments had started the seedlings in Treatment C were switched to long-day conditions and those in Treatment D to short-day.

The dry weights of the tops and of the roots were obtained for these seedlings in addition to the usual epicotyl length measurements. The soil was washed from the roots at the time of lifting. The seedlings were dried in a force-draft oven for three days at 77°C prior to weighing.

Test 5. Needle Pruning.

In this test seedlings were grown in the manner described earlier. The secondary needles were removed as soon as they appeared on 30 seedlings (six pots of five seedlings each). Thirty seedlings were left to develop naturally as checks.

The secondary needles were removed as soon as they could be recognized, usually when they were about 1/16 to 1/8 inches in length. This was accomplished by prying them out with a sharp, thin-bladed knife point. Considerable care was taken to keep injury to the seedling at a minimum and especially to keep from injuring the apical bud.

Once the secondary needles started appearing it required daily attention to keep them out.

When the seedlings were four months old, they were transplanted to two-gallon pots to give them more growing space. One third of the seedlings were lifted and measured at this time. Treatment was continued on the rest of the seedlings for over a year.

When the seedlings were six months old (in May) they were moved to a partially shaded greenhouse and were under natural day length. In November they were again returned to long-day (15 hour) conditions.

Test 6. Phloem Blocks.

Phloem blocks were installed on longleaf seedlings using the so-called "bark inversion" technique. In one experiment, eight-month-old longleaf seedlings which had been growing outside were moved into the greenhouse in October. Ten of these seedlings had a phloem block installed by making two cuts about a centimeter apart through the phloem to the xylem. The ring of bark was carefully removed and the xylem scraped to remove any connections. The ring of bark was then inverted and replaced on the seedling and taped in place. The top of the ring was about 0.5 cm below the cotyledon scars. The seedlings were then individually planted in plastic pots. Ten seedlings were also planted as controls.

Phloem blocks were also placed on ten small seedlings which were six weeks old.

Test 7. Oxygen Concentration.

In this test three pots of longleaf seed and three pots of slash seed were placed in a glass-sided box which was reasonably air tight. An equal number of seedlings of both species were kept outside the box as controls.

The oxygen content of the air was doubled by pumping in compressed air and oxygen from a storage bottle. The rate of flow was regulated with bubble counters. Air passed in at the rate of five bubbles to one of oxygen. A small circulatory fan inside the box kept the air well mixed. There was also a coil of copper tubing inside the box through which water passed to keep the temperature the same as outside. It was possible to keep the inside temperature within two degrees of the outside temperature. A 15-hour-day was maintained by suspending two fluorescent lights (40 watts each) two feet above the pots.

Test 8. Seed Treatment.

There were several separate tests in this category. In the first, longleaf seed directly out of storage was soaked in IAA, IBA, and NAA at a concentration of 0, 1, 10, 100 and 1000 ppm. The acids were put into water solution first dissolving them in a drop of five percent KOH. A stock solution was made up at the strongest concentration and then the other concentrations were obtained by dilution. The seeds were soaked for 24 hours in the solutions and then sowed in pots in the usual manner. About 15 seeds were sowed per pot and the seedlings were thinned to the five largest one month after germination. The test was installed in a randomized complete block design with three replications.

Another test was installed similar to the above using just the IAA. In this test the seeds were placed on moist filter paper in petri dishes for two days before giving them the 24-hour soak.

Test 9. Auxin Antagonists.

Longleaf seed was soaked in aqueous solutions of TIBA and NMSP at concentrations of 0.1, 1, 10, 0 and 100 ppm. Another solution, DCA, was prepared by putting 100 mg of DCA into a liter of water and letting it stand for two days at room temperature with frequent stirring. From this stock 1X, 0.1X, 0.01X and 0.001X dilutions were made.

After soaking the seed in these solutions for 20 hours the seeds were planted in pots. The planting was in a randomized complete block design with three replications.

In another phase of this test, longleaf seedlings were top dipped in the solutions for one minute before transplanting. Concentrations of TIBA and NMSP were 1, 10, 100 and 1,000 ppm and DCA at the same concentrations as used in the seed-soaking test. A detergent was added to a concentration of 0.2 percent. The planting was a randomized complete block design with three replications.

Test 10. Seed Treatment.

Longleaf seed was soaked in aqueous solutions of GA, IAA, 2,4,6-T and K at concentrations of 100, 20, and 4 ppm for 24 hours. After the soaking period they were sowed in vermiculite and placed under continuous light. Two days later half of the pots were moved into a dark room where they were held in continuous darkness except for short intervals of red light from a photographic safelight when the pots were being watered. There were no visible signs of germination at the time the seed were moved into the dark.

The seedlings grown in the dark were measured 27 days after treatment. The root length, primary needle length and cotyledon length was recorded.

The seedlings grown under continuous light were measured 45 days after treatment. The length of the primary needles, cotyledons and epicotyl length was recorded.

Test 11. Time of Application.

Longleaf seed were sowed in pots. Starting two weeks after germination a different lot of seedlings were sprayed with solutions of IAA every other week for a 10-week period. The concentrations of IAA were 0.1, 1.0, 10.0 and 100.0 ppm. The acid was put into solution by first dissolving it in a drop of five percent KOH. A stock solution was made at the highest concentration and other concentrations obtained by dilution. A detergent was added to the solutions to facilitate uptake of the IAA by the needles. This was at a 0.2 percent final concentration.

The seedlings were sprayed with a throat atomizer until runoff occurred. To keep the solutions from drying too rapidly all spraying was done in the evening and the greenhouse was sprinkled with water to raise the humidity.

The test was installed in the usual randomized complete block design with three replications.

Test 12. Repeat Spray.

Longleaf seed was sown in pots. Two weeks later the pots were thinned to five seedlings each. Starting at this time the seedlings were sprayed five times at bi-weekly intervals with IAA at concentrations of 0, 0.1, 1, 10 and 100 ppm. The solutions contained a detergent at 0.2 percent concentration.

Spraying was with a throat atomizer and seedlings were sprayed until runoff occurred. Spraying was done in the late evening and the greenhouse sprinkled with water prior to spraying to keep the solutions from drying too rapidly in the foliage.

The usual randomized complete block design was used with four replications per treatment.

Test 13. Growth Regulators.

Longleaf pine seed was sown in a germinating bed. Just before the primary needles started elongating the seedlings were lifted and the tops dipped in aqueous solutions of IAA, NAA, NOA and CPA in concentrations of 1, 10, 100 and 500 ppm plus an untreated control. Each solution contained a detergent at a concentration of 0.2 percent.

The seedlings were lifted and the tops dipped for one minute. They were then transplanted to pots. The planting was in a randomized complete block design with five replications.

Test 14. Kinetin.

Longleaf seedlings were top dipped in aqueous solutions of K in the manner described in Test 13. The concentrations used were 0.01, 0.1, 1 and 10 ppm, plus an untreated control. Detergent was also added to a concentration of 0.2 percent.

Planting was in a randomized complete block design with four replications.

Test 15. Rate Test.

Longleaf seed were germinated in a flat and transplanted to pots when they were one week old. When they were three weeks old GA, IAA, 2,4,6-T, TIBA and HT were applied to the apical meristem region at rates of 1, 10 and 100 ug per seedling. These chemicals were applied in an ethanol solution with a micro-pipette. Each seedling received the chemical in 10 ul of alcohol. Control seedlings received 0, 5 and 20 ul of ethanol. Kinetin was also applied at 1X, 0.1X and 0.01X a saturated ethanol solution.

This test was installed in a randomized complete block design with two replications. One replication of loblolly and one of slash seedlings were also included.

All of the seedlings were re-treated two weeks later and two weeks after this they were all treated again except the slash and loblolly seedlings were not treated with the high rate of 2,4,6-T.

Test 16. K and GA.

In this test longleaf, slash, loblolly and Sonderegger pine seed were germinated in a flat and transplanted to pots when they were two weeks old. The following treatments were used:

A. Untreated control.
B. Ethanol.
C. IAA.
D. Kinetin.
E. GA.
F. Kinetin + IAA.
G. IAA + GA.
H. Kinetin + GA.
I. Kinetin + IAA + GA.

These chemicals were applied in ethanol solutions to the apical meristem region. The first application of kinetin was made one week after potting. This application was at the rate of 20 ug in 10 ul of ethanol per seedling. The IAA and GA were applied at the rate of 10 ug per seedling one week later. Kinetin was applied again two weeks after the first application but at a rate of 10 ug per seedling. The IAA and GA were applied one week later. Two weeks after this the IAA and GA were again applied but not the kinetin.

The ethanol treated seedlings (B) received 10 ul of ethanol whenever any treatment was applied to the other seedlings.

This test was in a split-plot randomized complete block design with two replications.

Test 17. TIBA + GA.*

Longleaf seeds were germinated in a flat and transplanted when they were about two weeks old. The slash seedlings used in this test were handled in the same manner but were only one week older.

There were 9 treatments:

A. Ethanol control.
B. TIBA - 10.
C. TIBA - 100.
D. GA - 10.
E. GA - 100.
F. TIBA - 10 + GA - 10.
G. TIBA - 10 + GA - 100.
H. TIBA - 100 + GA - 10.
I. TIBA - 100 + GA - 100.

The first treatments were applied two weeks after potting. The numbers in the above treatments refer to the micrograms of acid applied per seedling. These were applied in 10 ul of ethanol. The TIBA + GA was applied simultaneously.

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* The gibberellic acid used in this test was supplied through the courtesy of the Eli Lilly Company.

The seedlings were re-treated two and five weeks after the first treatment.

There was a considerable alga growth on the surface of the soil in this test and the seedlings were only of fair vigor so the entire test was repeated in south Mississippi. In this second test the first treatment was applied when the seedlings were two weeks old. They were treated again when at three and four weeks old.

The gibberellic acid used in this second test was from an unknown source.

A randomized complete block design was used in both tests with three replications for the longleaf and two for the slash. Actually this was two experiments since a split-plot design was not used. However the slash and longleaf seedlings were placed side by side in the bed and were treated the same so a rough comparison can be made between the species even though it cannot be tested statistically.

Test 18. Hybrids.

Wind pollinated seeds from four natural hybrid pines (parents presumably longleaf and loblolly) were sown in a flat. The seedlings from these seeds had a range in hypocotyl length from four to 63 mm. Two groups of seedlings were separated out of this bulk lot. One group had hypocotyls which averaged 46 mm in length and the other group averaged 10 mm. These were transplanted to pots. There were five seedlings to a pot. The same number of the seedlings from each parent tree was put into each pot, i.e. two seedlings from one tree and one from each of the others.

Longleaf seeds were sown and transplanted at the same time as the hybrids.

Five treatments were applied to these seedlings:

A. Control (Alcohol).
B. TIBA - 5.
C. TIBA - 50.
D. GA - 5.
E. GA - 50.
F. GA - 5 + TIBA - 5.

The controls received 5 ul of ethanol each time the other seedlings were treated. The numbers in the above list of treatments refer to the micrograms of acid that was applied to each seedling in 5 ul of ethanol.

The hybrid seedlings with the long hypocotyls were treated two times at weekly intervals. The short-hypocotyl seedlings were treated three times at weekly intervals and the longleaf was treated five times in the same period. Treatments started when the seedlings were two weeks old.

The epicotyl lengths of the seedlings with the long hypocotyls were measured when they were five weeks old and the rest measured when they were six weeks old.

Statistically this was arranged as three separate tests, each in a randomized complete block design. Each category (longleaf, short-hypocotyl seedlings, long-hypocotyl seedlings) was treated in the same way as the others and they were arranged side by side on the greenhouse bench so generalizations can be made between them.

Test 19. Foliage Spray.

Longleaf seedlings were potted in the usual manner. When they were three weeks old they received the following treatments:

A. Untreated control.
B. Ethanol spray.
C. GA - 100.
D. TIBA - 20 ppm.
E. TIBA - 200 ppm.
F. TIBA - 2000 ppm.
G. TIBA - 20 ppm plus GA - 100.
H. TIBA - 200 ppm plus GA - 100.
I. TIBA - 2000 ppm plus GA - 100.

The ethanol and all TIBA was applied as a foliage spray. The TIBA was in an ethanol solution. The GA was applied to the apical meristem region in 10 ul aliquots of ethanol one week after the TIBA sprays. The GA was at the rate of 100 ug per seedling. The seedlings were all re-treated after two, four and six weeks except for the three combination treatments which did not receive the last two applications.

This was in a randomized complete block design with four replications.

Test 20. Weekly spray.

This test was conducted in the same manner as Test 19 with alcoholic solutions of TIBA being sprayed on the seedlings starting when they were three weeks old and weekly thereafter for four weeks. Concentrations of TIBA were 0, 20, 200, and 2,000 ppm.

The test was in a randomized complete block design with four replications.

Test 21. TIBA Rate.

Longleaf seeds were germinated in a flat and transplanted to pots when they were about two weeks old. Two weeks after potting TIBA was applied at rates of 0, 10, 100, 250, 500 and 1,000 ug per seedling. This was applied in 10 ul of ethanol to the apical meristem region with a micro-pipette. The seedlings were re-treated twice at bi-weekly intervals.

The test was installed in a randomized complete block design with four replications.

Test 22. Antagonists.

Longleaf seed was germinated in a flat and transplanted to pots when the seedlings were about two weeks old. One week after transplanting they were treated with trans-cinamic acid (TC), 2,4,6-T, and TIBA at rates of 10, 100, and 500 ug per seedling. Seedlings were also treated with DCA at a 1, 10, and 50 percent concentration. These were all in an ethanol solution. Ten microliters were applied per seedling. The seedlings were re-treated in two weeks and in four weeks with everything but the two highest concentrations of DCA. Six weeks after the first treatment, the seedlings were again treated with everything but the two highest DCA concentrations and all three 2,4,6-T treatments. The control seedlings were treated with 10 ul of ethanol at each of these dates.

This test was in a randomized complete block design with three replications.

Test 23. Synergists.

This test was conducted in south Mississippi using both longleaf and slash pine seedlings. Alcoholic solutions of indole or coumarin were applied to the apical meristem region at rates of 10, 100, and 500 ug per seedling in 10 ul aliquots. There was also an alcohol check and an indole plus GA and a coumarin plus GA treatment where 100 ug of each compound was applied. The first treatment was applied to seedlings which were two weeks old with re-treatment being made at four and five weeks.

Three replications of longleaf was used in a randomized complete block design. Two replications were used on the slash. This was actually two separate tests but since they were treated in the same manner, and side-by-side in the bed, a rough comparison can be made between them.

Test 24. Indole Carboxcylic Acid.

Longleaf seedlings were potted in the usual manner. When the seedlings were three weeks old they were treated with 0, 1, 10 and 100 ug of indole carboxcylic acid per seedling. This was applied in 10 ul aliquots of ethanol to the apical meristem region. The seedlings were re-treated when they were 5, 6, 7 and 8 weeks of age.

This test was in a randomized complete block design with four replications.

Test 25. Sugar Spray Test.

Longleaf and loblolly seeds were germinated in flats and the seedlings transplanted to pots when they were about two weeks old. They received the following treatments one week later:

A. Untreated control.
B. 70% alcohol.
C. 5% sucrose in 70% alcohol.
D. 5% glucose in 70% alcohol.
E. 15% sucrose in 70% alcohol.
F. 15% glucose in 70% alcohol.
G. 15% sucrose in water + detergent.
H. 15% glucose in water + detergent.

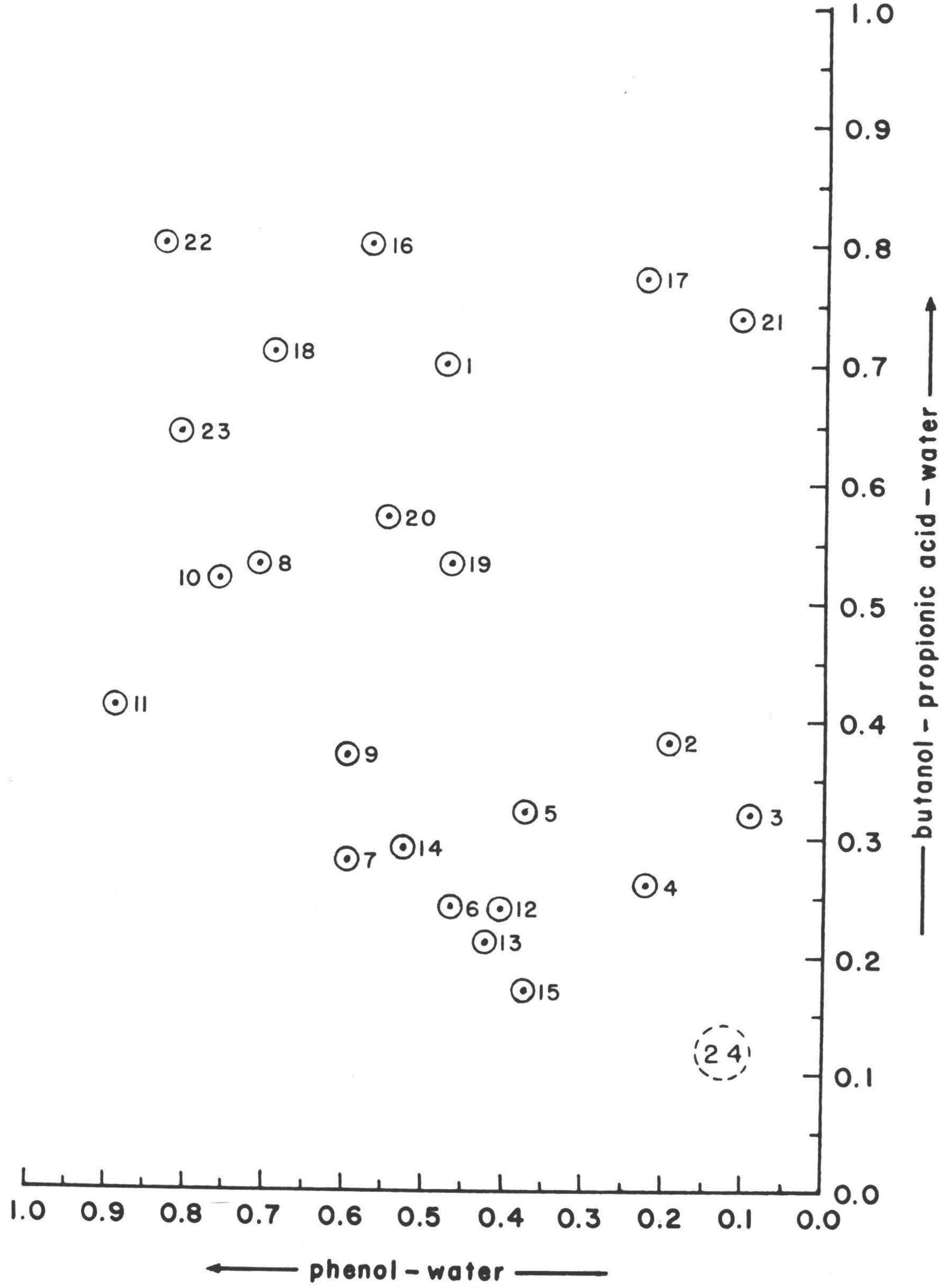
The above solutions were sprayed on the seedlings with a throat atomizer until runoff occurred. All spraying was done in late afternoon. The entire pot of seedlings was placed in a large plastic bag and left sealed until the next morning to prevent rapid drying of the solutions.

The seedlings were sprayed three times at bi-weekly intervals.

The test was installed in a split-plot design with three replications.

FIGURE 13. LOCATION OF ACTIVITY RESULTING FROM FEEDING LABELLED GLUCOSE AND SUCCINATE TO STEM TIPS OF LONGLEAF AND SLASH PINE SEEDLINGS

Numbers refer to numbers in Tables VIII and IX.



1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
butanol - propionic acid - water
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
phenol - water
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APPENDIX III

Rf's, Erlich Colors and Ultra-Violet Fluorescence of Some Indole Compounds

Compound	Rf		Erlich color	Ultra-violet fluorescence
	solvent A*	solvent B**		
B-Indolyl acetonitrile	0.76	0.79	Yellow	White (indistinct)
Ethyl 3-indoleacetate	0.88	0.82	Blue-purple	Indistinct
B-Indole-3-propionic acid	0.78	0.45	Blue-purple	Purple
Indole-3-n-butyric acid	0.83	0.52	Blue-purple	Purple
5-Methyl indole-2-carboxylic acid	0.59	0.48	Lavender	Light blue
Indole-3-acetic acid	0.64	0.40	Pink-purple	Lavender
Indole carboxcylic acid	0.82	0.30	Purple-pink	None
Indole acetamide	0.76	0.73	Green-blue	Lavender
Indole-3-aldehyde	0.88	0.82	Lavender	Blue-purple
Indole-3-acrylic acid	0.92	0.92	Red-peach	Orange
Indole-3-butyronitrile	0.91	0.86	Pink-purple	Light yellow
Indole-3-valerianic acid	0.81	0.55	Purple	Lavender
5-Hydroxy indoleacetic acid	0.14	0.23	Blue	Faint
5-Hydroxy tryptophan	0.04	0.16	Blue	Indistinct
L-Tryptophan	0.16	0.29	Red-purple	Lavender

* Isobutanol:methanol:water (8:1:1).
** Isopropanol:ammonia:water (8:1:1).

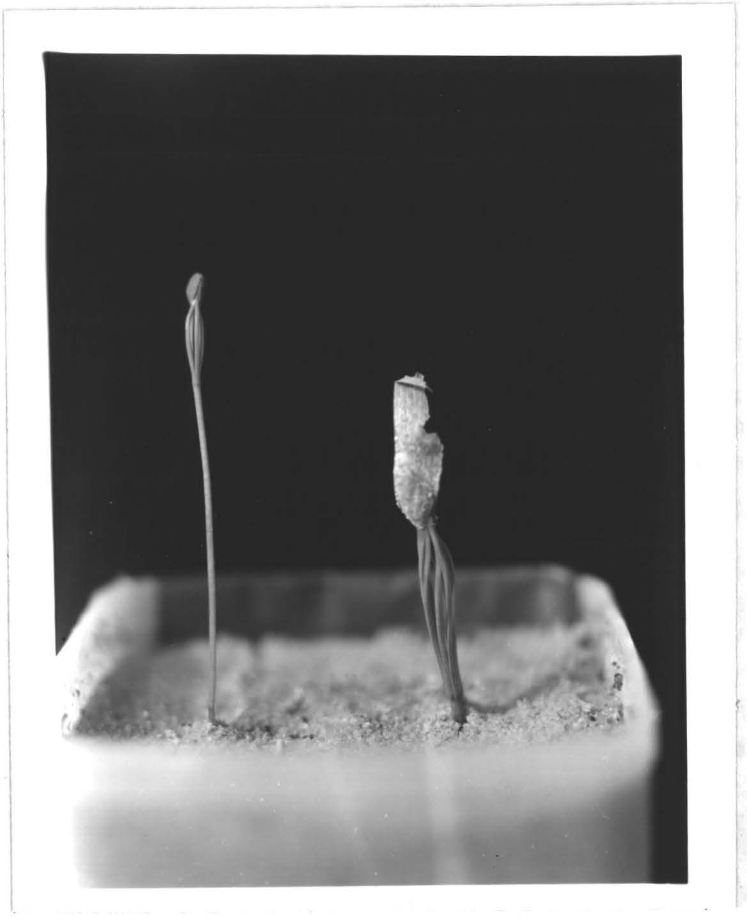

FIGURE 14. ONE-WEEK-OLD SLASH AND LONGLEAF PINE SEEDLINGS

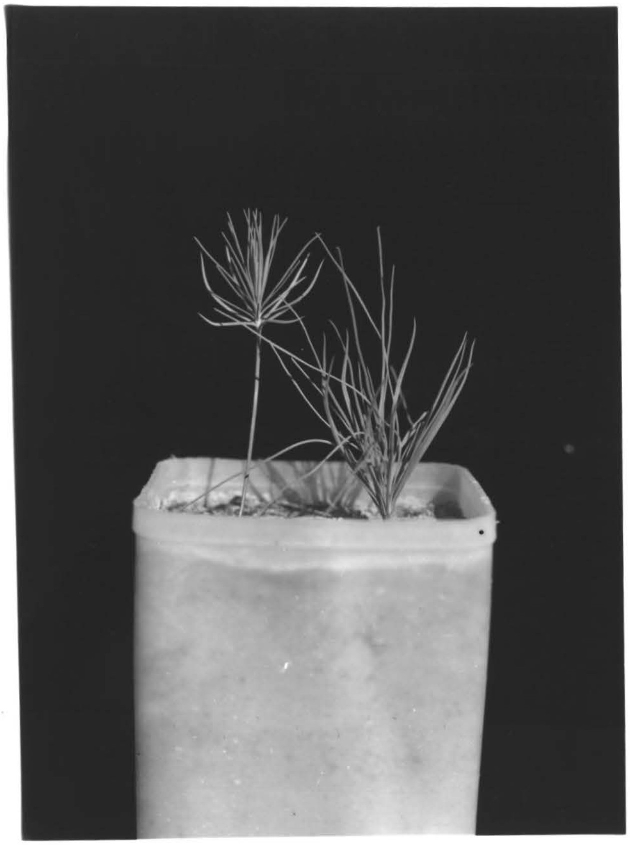

FIGURE 15. SIX-WEEK-OLD SLASH AND LONGLEAF PINE SEEDLINGS

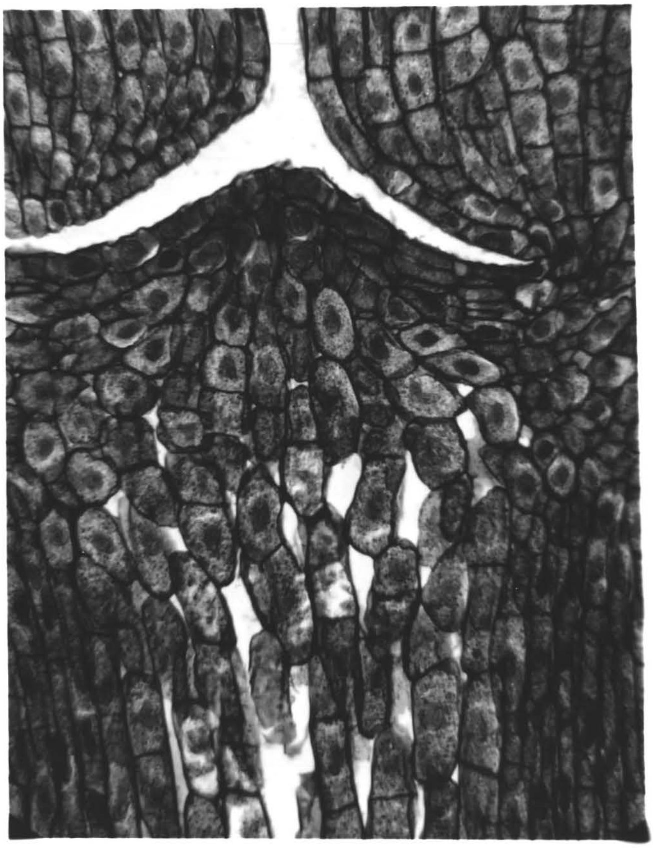

FIGURE 16. APICAL MERISTEM REGION OF A SLASH PINE EMBRYO

Eighty X magnification.

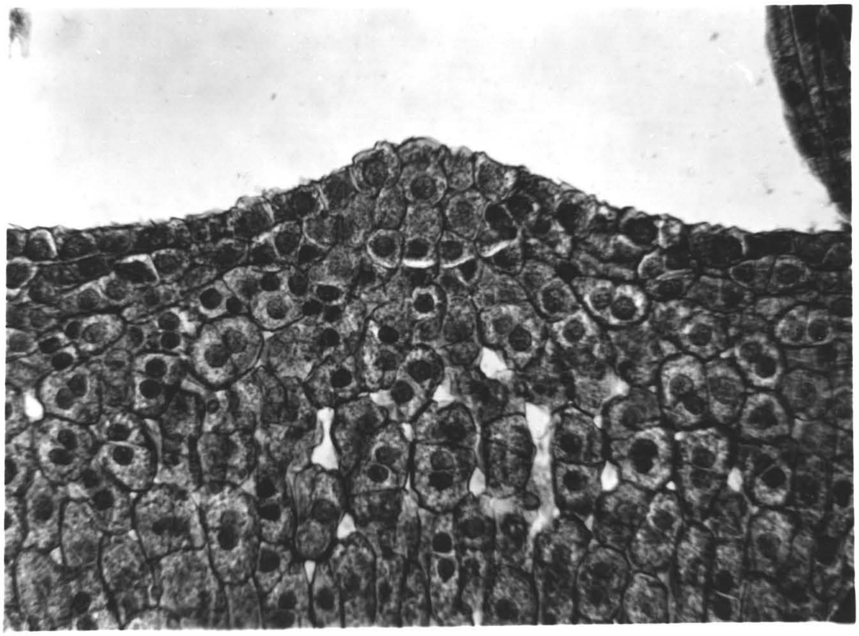

FIGURE 17. APICAL MERISTEM REGION OF A LONGLEAF PINE EMBRYO

Eighty X magnification.

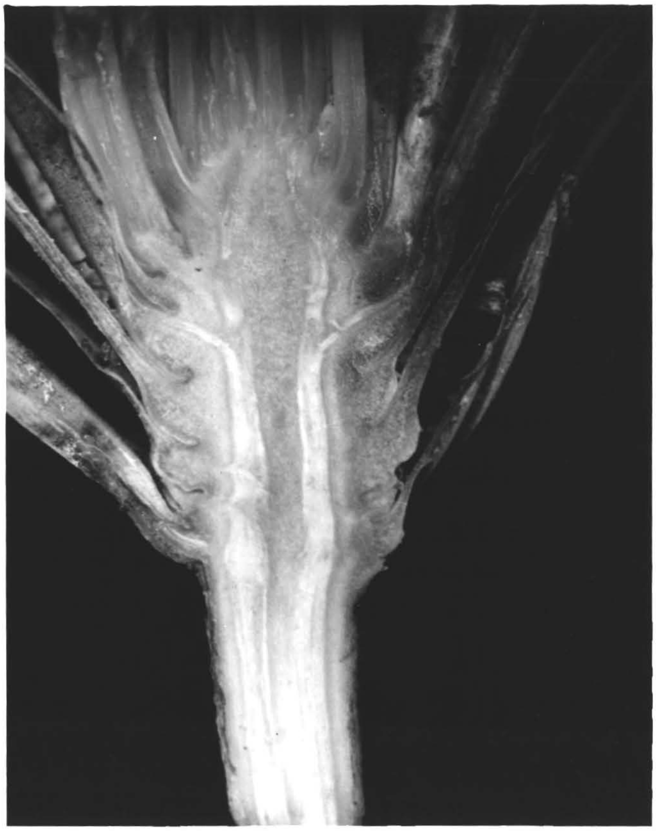

FIGURE 18. EPICOTYL OF A FOUR-MONTH-OLD LONGLEAF SEEDLING

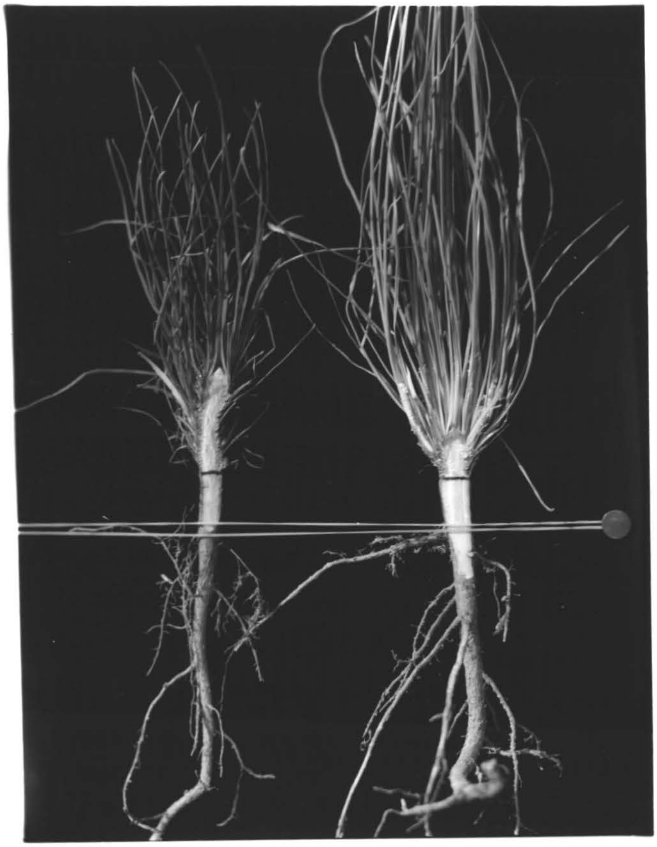

FIGURE 19. EFFECT OF THE REMOVAL OF SECONDARY NEEDLES ON LONGLEAF PINE EPICOTYLS

Left--secondary needles removed. Right--untreated control. See page 45 of text for discussion.

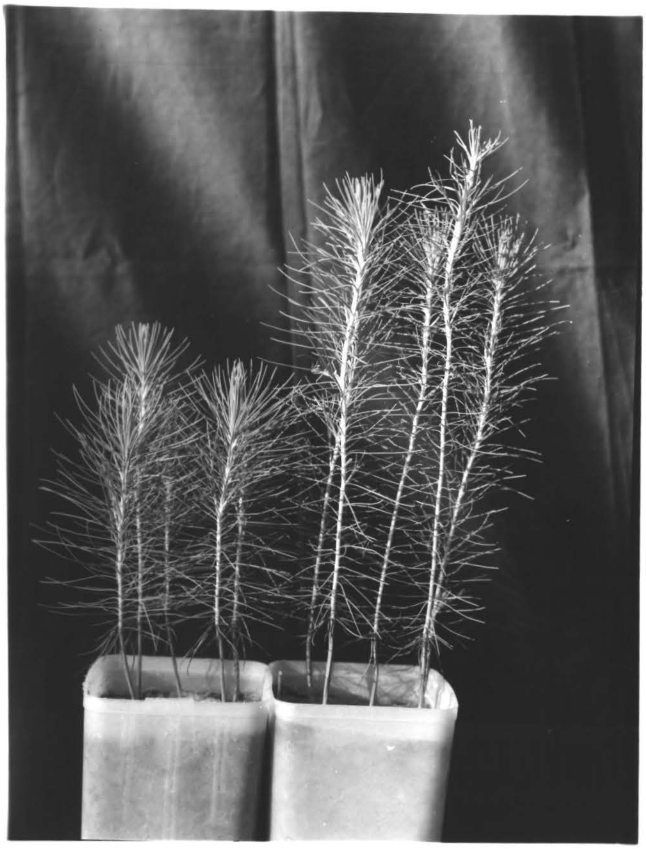

FIGURE 20. EFFECT OF GIBBERELLIC ACID ON SLASH PINE SEEDLINGS

Left--control. Right--treated.

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LITERATURE CITED

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BIOGRAPHY

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Publications :

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